Surprise in a small package: foregut metamorphosis in the marine ectoparasitic snail *Odostomia tenuisculpta* (Family Pyramidellidae)

by

Kathrina Harms
B.Sc., University of Victoria, 2016

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Supervisory Committee

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Abstract

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Members of the Pyramidellidae are ectoparasites that have highly unusual feeding habits relative to other heterobranch gastropods. Pyramidellid foregut anatomy is so complex that it is difficult to recognize homologous parts relative to other heterobranch gastropods, which is a necessary step in reconstructing evolutionary changes to the foregut developmental program. We investigated foregut development through metamorphosis and beyond in the pyramidellid *Odostomia tenuisculpta*. By examining sections of larval and post-velum loss stages, we conclude that the so-named acrembolic proboscis of this pyramidellid is actually an eversible oral tube and the piercing stylet is either a modified radular tooth or a jaw derivative. Much of the complex, multi-component foregut of the post-metamorphic stage is constructed during a 10-day period of explosive metamorphic morphogenesis. This stands in marked contrast to predatory neogastropods, where most components of the adult feeding system become extensively differentiated in the larval stage prior to settlement and metamorphosis.

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Acknowledgments

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1.0 Introduction

1.1 Evolvability, modularity and evolutionary novelties

Evolutionary developmental biology (evo-devo) is based around one central issue: to understand how developmental systems may bias or otherwise influence evolutionary change. Evolvability, or the "capacity of a developmental system to evolve" (Hendrikse et al. 2007; Kirschner 2013), refers to the developmental system's ability to create variation that natural selection can then work on. Development is therefore an integral part of any attempt to understand the evolutionary trajectory of homologous structures within a lineage of organisms (Hendrikse et al. 2007).

The importance of incorporating development into an understanding of evolutionary processes has been based on several important factors. First, research has shown that the way that development is organized within an organism can bias the type, amount, and direction of phenotypic variation that is created (Alberch 1980). Second, an understanding of development can help address the evolutionary origin of novelties, which the Modern Synthesis with its sole emphasis on selection, has failed to explain (Moczek et al. 2011; Kirschner 2013). Third, an understanding of developmental organization might help explain how complex, multi-component systems can evolve at all. How can any one part of a morphological complex that generates a functionally essential component of the body plan undergo change, without fatally compromising development and functional integrity of the final product (Raff 1996)?

A developmental mechanism that has been suggested to facilitate evolvability and the creation of evolutionary novelties within species is a modular organization of development. The term 'modularity' refers to a concept of biological organization in which some elements are grouped together into integrated subsets, which are less integrated with other subsets (Klingenberg 2008). Elements within a module can be as small as nucleotides in a molecule of RNA or as large as organ-level morphological characters (Wagner et al. 2007). Developmental systems can be subdivided into separate modules, where each module develops independently relative to other modules (Raff

1996; Von Dassow and Munro 1999; Bolker 2000). Developmental modules are likely under the control of discrete gene regulatory networks causing elements within a module to develop together in an integrated fashion (Wagner and Altenberg 1996; Wagner et al. 2007).

The independence of developmental modules may generate novelties through heterochrony, because a module can be offset in space or time relative to other modules without deleterious consequences (Gould 1977). Furthermore, innovative types of developmental modules can allow development to circumvent constraints that are placed on one life-history stage by another. Otherwise, evolutionary change of one life-history stage may be constrained by previous or subsequent life-history stages (Alberch 1987). For example, some insects possess holometabolous development, in which a pupal stage is incorporated that has allowed for the evolution of a great diversity of forms. The pupal stage is a transitional stage between the larval and adult stages (Belles 2011), in which massive morphological changes occur. Due to this period of massive structural reorganization, both the larval and adult body plans have been free to diverge radically because neither stage is constrained by the functional needs of the other.

In the case of the gastropod foregut, Page (2000, 2002, 2005, 2011), Parries and Page (2003), Hookham and Page (2016) and Page and Hookham (2017) have all suggested that the foregut is made of two developmental modules and that separation of these modules (both temporal and spatial) may have facilitated the emergence of diverse and complex post-metamorphic foregut types. The dorsal module consists of the larval esophagus and becomes the dorsal food channel of the post-metamorphic foregut (only in herbivorous gastropods). The ventral module originates from stem cells embedded in the ventral larval esophagus, which proliferate and form a ventral out-pocketing that eventually differentiates into the post-metamorphic buccal cavity and radular sac (Fretter 1969; Page 2000, 2002, 2005, 2011; Hookham and Page 2016). The neogastropods are a group of gastropods in which temporal and spatial dissociation of their two foregut modules may have promoted the evolvability of the post-metamorphic foregut, because the ventral module separates almost completely from the dorsal module during development. A very narrow connection is present, but most of the ventral out-pocketing is isolated. Therefore, the ventral module (post-metamorphic foregut) can develop into a

wide range of diverse morphologies (Ponder 1973; Kantor 1996) without interfering with larval feeding (Page 2011).

In this thesis, I examined how evolution of development has resulted in the generation of a highly specialized and modified adult foregut in a gastropod species that begins life as a larva that feeds on a different type of food using different feeding structures.

1.2 Life histories of gastropods

Life-histories of gastropods are variable depending on the species and can be categorized according to: presence or absence of a larval (dispersive) stage, presence or absence of metamorphosis and the source of larval nutrition (Bonar 1978). The life-history patterns of gastropods with a larval stage must be considered in evolutionary and developmental studies because much of the larval body in gastropods is typically carried into the juvenile stage to form a large part of the juvenile body; as such, both stages are important (Page 2009).

Two main life-history patterns exist for gastropods: indirect and direct (Figure 1). A species with indirect development is first present as an embryo (usually but not always encapsulated), which develops into a free-living veliger larva enabling larval dispersal, and then undergoes metamorphosis to become a juvenile. Gastropods with indirect development can be further subdivided into planktotrophic (feeding) or lecithotrophic (non-feeding) larvae; planktotrophs feed on microalgae that are present within the water column whereas lecithotrophs are usually provisioned with albumin, maternal yolk or both within the egg capsule (Fretter and Graham 1994) and therefore do not need to feed. Compared to indirect developers, embryos of species with direct development undergo development to the functional juvenile stage while encased entirely within an egg capsule, therefore limiting dispersal capabilities. Most direct developing gastropods pass through a stage in which veliger characteristics are recognizable; these are referred to as veliger-like embryos (Fretter and Graham 1994), because they are not planktonic.

After veliger larvae and veliger-like embryos have undergone sufficient development, they proceed through metamorphosis and become juveniles. Metamorphosis involves the loss of distinctly larval characters (e.g. velar lobes) followed by the emergence of distinctly juvenile structures (e.g. proboscis) (Hadfield et al. 2001). However, the type of life-history in which direct developers become juveniles without passing through a veliger-like stage is called an ametamorphic life-history, because there is no metamorphic reorganization of the body during development.

The generalized veliger body plan includes a foot with an operculum, a larval shell (protoconch) that is calcified, and velar lobes. The velar lobes are made up of two-flattened head extensions with bands of cilia that are used for feeding and swimming. Encapsulated veligers, although they do not capture microalgae, still have ciliated velar lobes; the lobes allow the veliger-like embryo to rotate within the egg capsule, potentially aiding in albumin uptake (Rivest 1992) and gas exchange (Hunter and Vogel 1986). The bands of cilia that run along the periphery of the velar lobes are called the prototroch and metatroch (Strathmann and Leise 1979). The cilia of the prototroch and metatroch beat in opposite directions (Thompson 1959; Strathmann and Leise 1979). As they beat towards one another, the two bands create a feeding current to capture food particles (Strathmann and Leise 1979). The captured particles are then transported along a food groove, located between the two ciliary bands, to the mouth.

Within the Caenogastropoda, the system of adult feeding has evolved within an indirect life-history. Although some metazoans have a post-metamorphic body that develops mostly independent from the larval form (e.g. echinoderms), the (caeno)gastropod post-metamorphic juvenile structures are mostly built from elaborations of the larval body (Page 2002, 2005). Nevertheless, the functional requirements of both the larva and juvenile must be accommodated before and after metamorphosis if any evolutionary change is to occur (Page 2002). Two important requirements must be met if novel feeding strategies are to occur in adult gastropods: microalgae capture in larvae must be unencumbered during larval development and the juvenile foregut must be ready for feeding soon after metamorphosis is complete (Fretter 1969; Page 2000; Hadfield 2001). Larvae need a mechanism to collect and consume microalgae, yet the juvenile form can have a variety of different feeding requirements and structures depending on the

species. As such, specialized juvenile foregut morphology can be created if and when the functional constraints of the larva can be by-passed, either via novel developmental mechanisms (Alberch 1987) or by simply losing the functional larval stage (Wake 1966).

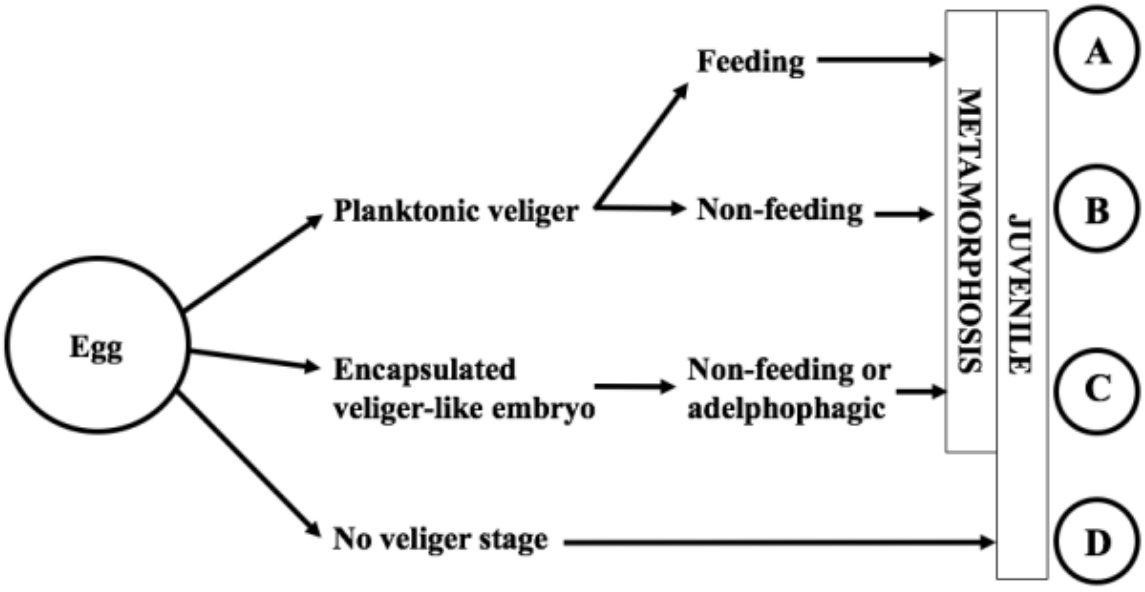

Figure 1. Major life-history patterns found in the Gastropoda; Ⓐ Indirect planktotrophic veliger, Ⓑ Indirect lecithotrophic veliger, Ⓒ Veliger-like embryo, Ⓓ No veliger stage (does not undergo metamorphosis). Adapted from Bonar 1978.

1.3 Gastropod feeding systems

The phylum Mollusca includes a huge diversity of animals. It is the second largest metazoan phylum and contains seven to eight classes and an estimated 200,000 extant species (Ponder and Lindberg 2008). The largest group of molluscs is the Gastropoda, which have radiated into a variety of environments and possess huge diversity in regard to their physiology, behavior and morphology. As gastropods invaded a variety of different habitats within the ocean, freshwater bodies, and even terrestrial environments, different feeding strategies evolved to exploit available resources in each biome (Ponder and Lindberg 1997; Ponder et al. 2008).

The most basal gastropod clades, as suggested by the phylogenetic hypothesis of Zapata et al. (2014), are the Patellogastropoda (true limpets) and the Vetigastropoda, both of which show the pleisiomorphic condition of herbivorous grazing. Some members of the Heterobranchia, Neritimorpha and Caenogastropoda have also retained the ancestral condition of herbivorous grazing (Ponder and Lindberg 1997). The foregut of a typical herbivorous gastropod begins anteriorly with a mouth that opens into an oral (buccal) tube that subsequently expands as the cuticle-lined buccal cavity. The buccal cavity receives the ducts of the salivary glands and leads posteriorly into the esophagus. From a ventral out-pocketing on the wall of the buccal cavity extends the radular sac, which secretes a ribbon of recurved teeth, commonly known as a radula (reviewed by Fretter and Graham 1994). From the radular sac the radula emerges and extends over the ventral floor of the buccal cavity towards the mouth. The radula is supported by radular (odontophoral) cartilages and complex muscles produce rhythmic radular movements; it is used by the gastropod to scrape detritus and algae off substrates (Fretter and Graham, 1994; Ponder and Lindberg 1997). Grouped together, the radular sac, buccal cavity, muscles and cartilages constitute the buccal mass. The ciliated dorsal food channel, which is delineated by a pair of dorso-lateral folds, runs down the buccal cavity along its dorsal midline (Haszprunar 1988; Salvini-Plawen 1988) and down the esophagus to the mid-esophageal gland.

Although gastropods that are predatory possess several similarities to ancestral herbivorous grazers, they also have some specialized features and an increasingly

complex feeding system. For example, the proboscis of gastropod predators requires an elongation of specific foregut components; the additional length comes from either an elongate anterior esophagus (Kantor 1996; Page 2011) or an elongation of the buccal tube (Kantor 1996). Predatory feeding also utilizes a valve structure, the valve of Leiblein, which may prevent regurgitation of ingested food in predatory gastropods with a pleurembolic proboscis (Graham 1941; Kantor 1996). Certain glandular structures appear to be adaptations to a predatory life history in order to deal with the requirements of capturing and digesting prey tissues (Kantor 1996). For example, the gland of Leiblein (derived from the mid-esophageal gland) may initiate digestion (Andrews and Thorogood 2005), accessory salivary glands in *Nucella* (dog whelks) may have a paralytic function (Andrews 1991) and the venom gland in conoideans (Gastropoda, Neogastropoda), which is also derived from the mid-esophageal gland (Page 2012), secretes neurotoxic peptides to paralyze prey (Olivera 2006).

An important question regarding the evolution of gastropod feeding (especially in the caenogastropods) is how they transitioned from herbivorous to predatory feeding in a two-phase life-history that begins with a herbivorous larva. As described below, comparative developmental studies provided answers to this evolutionary question.

1.3.1 Foregut development in gastropods

Herbivorous Caenogastropods

Many caenogastropods have retained herbivorous grazing as their mode of feeding into the adult stage; one such example is *Lacuna vincta*. The developing post-metamorphic foregut begins as a proliferation of stem cells on the ventral side of the distal larval esophagus (Page 2000). At approximately 20% to 30% of completed larval development, the cells begin to form an out-pocketing (Page 2000). Later in larval development the ventral out-pocketing differentiates into the specific structures of the post-metamorphic foregut, including the buccal cavity, radular sac, and salivary glands. Odontophoral cartilages and muscles become recognizable beneath the floor of the buccal cavity. As the prospective juvenile foregut develops from the ventral out-pocketing underneath the larval esophagus, the larval esophagus remains functional and continues as a passage for microalgae (Fretter 1969; Page 2000). At metamorphosis, the radular sac becomes partially uncoiled, moves posteriorly and the odontophoral cartilages and musculature enlarge and complete differentiation (Fretter 1969; Page 2000). The larval esophagus becomes reduced in size but remains present throughout the juvenile/adult stages as a ciliated channel that runs along the roof of the buccal cavity (Page 2000). This is called the ciliated dorsal food channel (Fretter and Graham 1994).

Predatory Caenogastropods

Although herbivory has been suggested as the ancestral mode of feeding in gastropods (Ponder and Lindberg 1997), certain groups of extant caenogastropods have utilized an extensive variety of nutritional resources by acquiring various different feeding strategies. Some novel feeding strategies include suspension feeding, predation of whole prey items, parasitism (Taylor et al. 1980), carnivorous grazing and scavenging (Ponder and Lindberg 1997). Diversification of the feeding system among predatory caenogastropods has been the hallmark of much of their diversity (Ponder 1973). Whereas grazing herbivorous gastropods use a radula to rasp algal films, some predatory

caenogastropods (more derived clades) use a muscular proboscis that protrudes from the anterior head region in order to reach new food sources or food that is difficult to collect (Taylor et al. 1980; Kantor 1996). Proboscis morphology is highly diverse within the Caenogastropoda; four to five different types have been identified based upon how they operate and their different retractor muscles (Golding et al. 2009).

The Neogastropoda are caenogastropods that arose during the Cretaceous (Ponder 1973; Taylor et al. 1980) and are highly derived, predatory caenogastropods that have an extremely complex foregut and well-developed proboscis (Kantor 1996). Members of this group have evolutionary novelties in their developmental program, compared to that of less morphologically derived gastropod groups (Page 2005, 2011, 2012). Neogastropods are exceptional for many reasons, especially their diversity of derived radular teeth. Traditionally radular teeth are secreted in multiple rows attached to a base ribbon made of chitin. Contrary to this, members in the family Conidae (superfamily Conoidea) have radular teeth that are present in the form of individual harpoons that shoot into prey (Schulz et al. 2004) in order to inject neurotoxins. These hollow harpoons are attached to the buccal mass only by a basal ligament (Kohn et al. 1972; Kantor and Taylor 1991).

Studies on the neogastropod *Nassarius mendicus* (Buccinoidea) revealed an increased spatial uncoupling between the larval esophagus and the prospective post-metamorphic foregut (ventral out-pocketing) (Page 2000, 2005). The hatching neogastropod larval foregut is much like that in larvae of herbivorous gastropods, in that it is a simple ciliated tube. A semi-isolated ventral out-pocketing (future juvenile/adult foregut) develops beneath the larval esophagus, with a narrow connection located at the posterior of the buccal cavity (Page 2000, 2005). In *N. mendicus*, the ventral out-pocketing gives rise to not only the buccal cavity and radular sac, but also the entire anterior esophagus that will extend down the elongate, post-metamorphic proboscis and the valve of Leiblein (Page 2000, 2005). At the initiation of metamorphosis, the larval mouth is paved over by epithelial cells and the larval esophagus is destroyed. The buccal cavity subsequently breaches through the body wall, and a new juvenile/adult mouth is formed (Page 2000, 2005).

Page (2000) suggested that the elongate adult anterior esophagus and associated proboscis structure arose after the emergence of two evolutionary novelties. One novelty was the formation of a new mouth during metamorphosis, likely allowing for the transition from herbivorous to predatory feeding (Page 2000). The second novelty was the incorporation of the prospective anterior esophagus and valve of Leiblein into the ventral out-pocketing, which previously generated only the buccal cavity and radular sac. Indeed, the anterior esophagus and valve of Leiblein of neogastropods appear to be a posterior elaboration of the buccal cavity, as previously suggested by Ponder (1973). These novelties (new developmental processes) helped accommodate the evolution of the elongate proboscis, which in turn helped facilitate the radiation of predatory gastropods as previously unavailable resources subsequently became available (Ponder 1973) and developmental constraints were bypassed.

Euthyneurans

There have been a number of studies, ultrastructural and histological, providing information on euthyneuran morphogenesis and the development of euthyneuran veligers, although these have largely concentrated on the group traditionally known as the "Opisthobranchia" (Thompson 1958, Tardy 1970, Thiriot-Quiévreux 1970, 1977, Bonar and Hadfield 1974, Kriegstein 1977a, 1977b, Bickell and Chia 1979, Bickell et al. 1981, Bickell and Kempf 1983, Tsubokawa and Okutani 1991, LaForge and Page 2007). However, there is little detailed information on foregut development of euthyneuran heterobranchs. Initial knowledge about the morphogenesis of the veliger stage of "opisthobranchs" came from species in which the larva is lecithotrophic, where larvae undergo metamorphosis after a very brief post-hatch period (Thompson 1958; Bonar and Hadfield 1974) or within an egg capsule (Tardy 1970). Development of the planktotrophic larva of the nudipleuran *Doridella steinbergae* (Lance, 1962), was investigated by Bickell and Chia (1979) and Bickell et al. (1981), in order to document the morphogenesis of its digestive system. Although these studies provided detailed information on larval gut morphology for planktotrophic opisthobranch veligers,

compared to those of lecithotrophs, and on the process of stomach metamorphosis, little information was provided on metamorphic changes of the foregut.

1.4 Pyramidellidae (Euthyneura; Panpulmonata)

Pyramidellids are usually tiny gastropods, most about 5 mm and some less than 3 mm in shell length (Schander et al. 2003), and all are ectoparasitic predators on other invertebrates, mainly bivalves and annelids (Fretter and Graham 1949; Robertson and Mau-Lastovicka 1979; Wise 1996; Schander et al. 2003). The family is located within the gastropod subclass Heterobranchia, and is subdivided into four subfamilies: Odostomiinae, Pyramidellinae, Turbonillinae and Syrnolinae (Bouchet et al. 2017). The family includes 350 genera containing more than 6000 species (Schander et al. 2003) and pyramidellids are globally distributed within brackish and benthic marine communities (Peterson 1998). The vast species richness of the family has been attributed to the evolution of an ectoparasitic adult stage with a proboscis; this proboscis is assumed to have increased accessibility to a variety of food sources not previously available (e.g. bivalves and tube-dwelling polychaetes) and contributed to the switch from herbivory to a carnivorous lifestyle (Dinapoli et al. 2011). Parasitic feeding has also involved a great deal of modification to the digestive system, including a piercing stylet to penetrate the body wall of a host, a pumping mechanism to withdraw host fluids, elongation of many foregut components, and simplification of the stomach (Fretter and Graham 1949; Wise 1993; Peterson 1998).

According to Fretter and Graham (1949) each member of the Pyramidellidae has its own specialized habitat and is host-restricted. For example, *Turbonilla elegantissima* (Montagu) ploughs through surface sediment to seek out tentacles of the terebellid worms, *Audouinia tentaculata* or *Amphitrite gracilis* (Fretter 1951). More recent research has suggested that only a few pyramidellid species are host-specific and specificity depends on numerous external and internal factors. *Boonea impressa* can feed on an array of invertebrate species (Robertson and Mau-Lastovicka 1979). For example, juvenile *B. impressa* have been found on oyster spat and *Crepidula plana* (the slipper limpet) (Powell et al. 1987). Juvenile *B. impressa* were also found on adult *Boonea*, potentially because their proboscis apparatus during the juvenile stage was too short to feed on oyster spat (Powell et al. 1987). *Boonea* species have also been known to parasitize *Crepidula* species other than *C. plana* (Robertson 1957) and *B. seminuda* fed on *C.*

fornicata as well as four other common molluscs (Robertson and Mau-Lastovicka 1979). From these results, the genus *Boonea* is clearly not host-specific in its feeding practices, and may even represent a species that can undergo an ontogenetic switch in prey choice. The pyramidellid *Fargoa dianthophila*, however, is a host-specific specialist on the serpulid polychaete *Hydroides dianthus*, as it and another species, *F. bartschi*, are in direct competition with each other, both for habitat space and food (Robertson and Mau-Lastovicka 1979). According to Robertson and Mau-Lastovicka (1979), generalist feeding is the more primitive condition; pyramidellids were originally not host-specific.

Like other pyramidellids, *Odostomia tenuisculpta* (Carpenter, 1864) is an ectoparasitic snail that collects nutrients from its invertebrate host with a so-called acrembolic proboscis that pierces the host and sucks out blood or even tissue with its buccal pump (Maguire and Rogers-Bennett 2013). *Odostomia tenuisculpta* has been found along the west coast of North America from Alaska to California (Abbot 1974), where it parasitizes abalone, scallops, mussels, clams and chitons (Harbo et al. 2012, 2013; Maguire and Rogers-Bennett 2013).

1.4.1 Phylogenetic controversies

Phylogenetic controversies associated with the Pyramidellidae have been superimposed on a larger controversy concerning the higher level taxonomy of the Gastropoda. For over 100 years, the Gastropoda was subdivided into three subclasses, the Prosobranchia, Opisthobranchia and the Pulmonata, as originally advocated by Milne-Edwards (1848) (Figure 2A). Although Spengel (1881) subsequently combined the Pulmonata and Opisthobranchia into a single clade, the Euthyneura, Thiele's (1931) influential “Handbuch der Systematischen Weichtierkunde” retained Milne-Edwards’ three gastropod subclasses and the Pyramidellidae, together with several other groups of ectoparasitic gastropods that lacked a radula, were placed within a group of prosobranchs. Towards the end of the last century, Haszprunar (1985, 1988) and Ponder and Lindberg (1997) initiated a major taxonomic revision of the Gastropoda, in which the traditional three major clades of gastropods were extensively reorganized (Figure 2B). Thus, the polyphyletic ‘Prosobranchia’ was disbanded and most of its members were distributed

into the Patellogastropoda, Vetigastropoda, Neritimorpha, and Caenogastropoda (Ponder and Lindberg 1997). The Opisthobranchia and Pulmonata underwent an even more extensive and still ongoing revision. Haszprunar (1985) resurrected Spengel's (1881) Euthyneura (combining the Opisthobranchia and Pulmonata), which he preferred to call Pentaganglionata under the argument that five ganglia along the visceral connective of the nervous system is an ancestral trait for this group, whereas euthyneury (absence of a torsional twist in the visceral connective) is a convergent trait. He also withdrew the Pyramidellidae and two other groups from the Prosobranchia and placed these as the sister group to the Euthyneura, which collectively constituted the Heterobranchia.

Early evidence for the hypothesis that pyramidellids were more closely allied with 'opisthobranchs' than with 'prosobranchs' was presented by Thorson (1946), who drew attention to the hyperstrophic larval shell coil of the pyramidellid, *Odostomia sp.*, as documented by Lebour (1932). Larval hyperstrophy is uniquely present in both opisthobranchs and pulmonates with a free-living larval stage. Subsequently, Robertson (1985) pointed out that pyramidellids and opisthobranchs also exhibit chalazae interconnecting egg capsules within the egg mass and the larvae have a pigmented mantle organ. The pigmented mantle organ (pmo) is a large glandular structure, which may possess a repugnatorial function, located inside the mantle cavity near the anus in larval (and adult) pyramidellids and opisthobranchs; it has previously been called a larval kidney and even an anal gland (Robertson 2012). Other similarities between larval pyramidellids and larval opisthobranchs include: lack of eyespots at hatching (Collin and Wise 1997) and hyaline rods embedded in the gastric shield of the stomach (Thompson 1959; Ponder and Lindberg 1997). Adult pyramidellids possess several features that unite them with the heterobranchs. They have a hermaphroditic sexual system and they lack a pectinate gill (ctenidium); instead, the mantle cavity has ciliated strips located dorsally and ventrally that bring in and circulate water (Wise 1996). Water flows into the mantle cavity over the top of the head then exits near the right-hand tentacle (Peterson 1998).

Schander et al. (2003) used 16S rDNA from 32 different species in order to test the monophyly of *Odostomia* and *Turbonilla.* Not only was the monophyly of Turbonillinae rejected with significance, the monophyly of *Odostomia* was suspect and hence the authors suggested that many taxa within the Pyramidellidae may also be non-

monophyletic (Schander et al. 2003). However, a subsequent study (Dinapoli et al. 2011) used both16s rDNA and 18s rDNA and included a greater number of species from the Turbonillinae and Odostomiinae to test whether pyramidellids are monophyletic and to identify the sister group within the Heterobranchia. They found, with high statistical support, that Pyramidellidae is monophyletic, as are the subfamilies Turbonillinae and Odostomiinae, contrary to the findings of Schander et al. (2003).

Subsequent molecular phylogenetic studies have suggested that, rather than including pyramidellids within the paraphyletic 'Lower Heterobranchia' as advocated by Haszprunar (1985, 1988), Pyramidellidae should be included within the Euthyneura (Grande et al. 2004; Dinapoli and Klussmann-Kolb 2010) (Figure 3). Most recently, Jörger et al. (2010), Kocot et al. (2013), and Wägele et al. (2014) suggested that Pyramidellidae is a monophyletic group within the Panpulmonata, a group that also contains traditional 'pulmonate' groups and two groups formerly within the 'Opisthobranchia', the Sacoglossa and the Acochlidia (Figure 4).

Molecular studies have provided robust support for the placement of pyramidellids within euthyneuran heterobranchs, but the sister group of the pyramidellids remains controversial. Grande et al. (2004, 2008), using complete mitochondrial genomes to assess relationships within the Gastropoda, placed the pyramidellids as sister to *Onchidella celtica* (Onchidioidea), although taxon sampling was limited. Subsequent studies using data from 18S, 28S, 16S, and cytochrome oxidase I genes, found the pyramidellids to be associated with Amphiboloidea (Klussmann-Kolb et al. 2008) and Dinapoli and Klussmann-Kolb (2010) and Jörger et al. (2010) found that the pyramidellids were closely related to Amphiboloidea ('Pulmonata') and Glacidorboidea ('Pulmonata'). Alternatively, the phylogeny of Dayrat et al. (2011), which was also based on nuclear (complete ribosomal 18S) and mitochondrial markers (partial sequences of ribosomal 16S and cytochrome oxidase I), placed the pyramidellids as sister to the Lymnaeoidea.

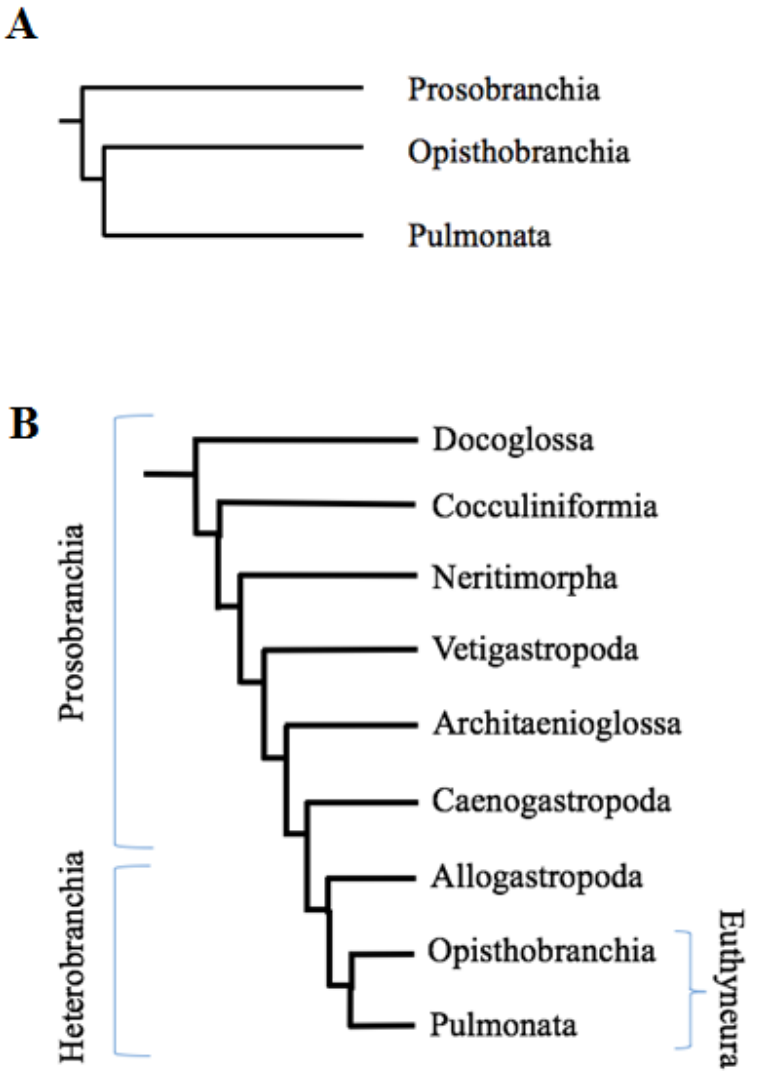

Figure 2. A. Original three Gastropod groups proposed by Milne-Edwards (1848). **B.** Major taxonomic revision of the gastropod groups; Pyramidellidae were included within the Allogastropoda. Adapted from Haszprunar (1985, 1988) and Ponder and Lindberg (1997).

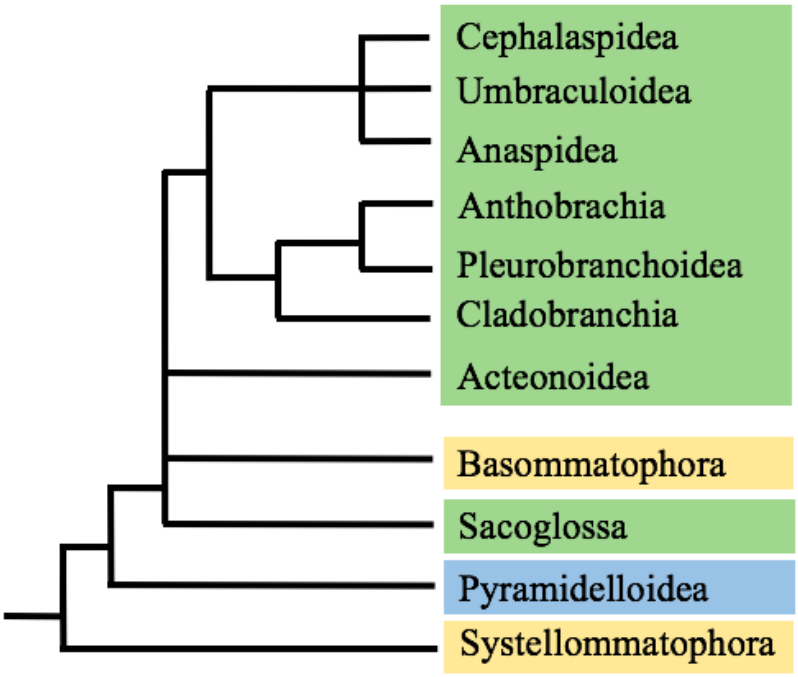

Figure 3. Phylogeny based on mitochondrial genome data from Grande et al. (2004); some taxa were partly combined for efficiency. Adapted from Wägele et al. (2014). Blue represents traditional lower heterobranchs, green represents traditional opisthobranchs and yellow represents traditional pulmonates.

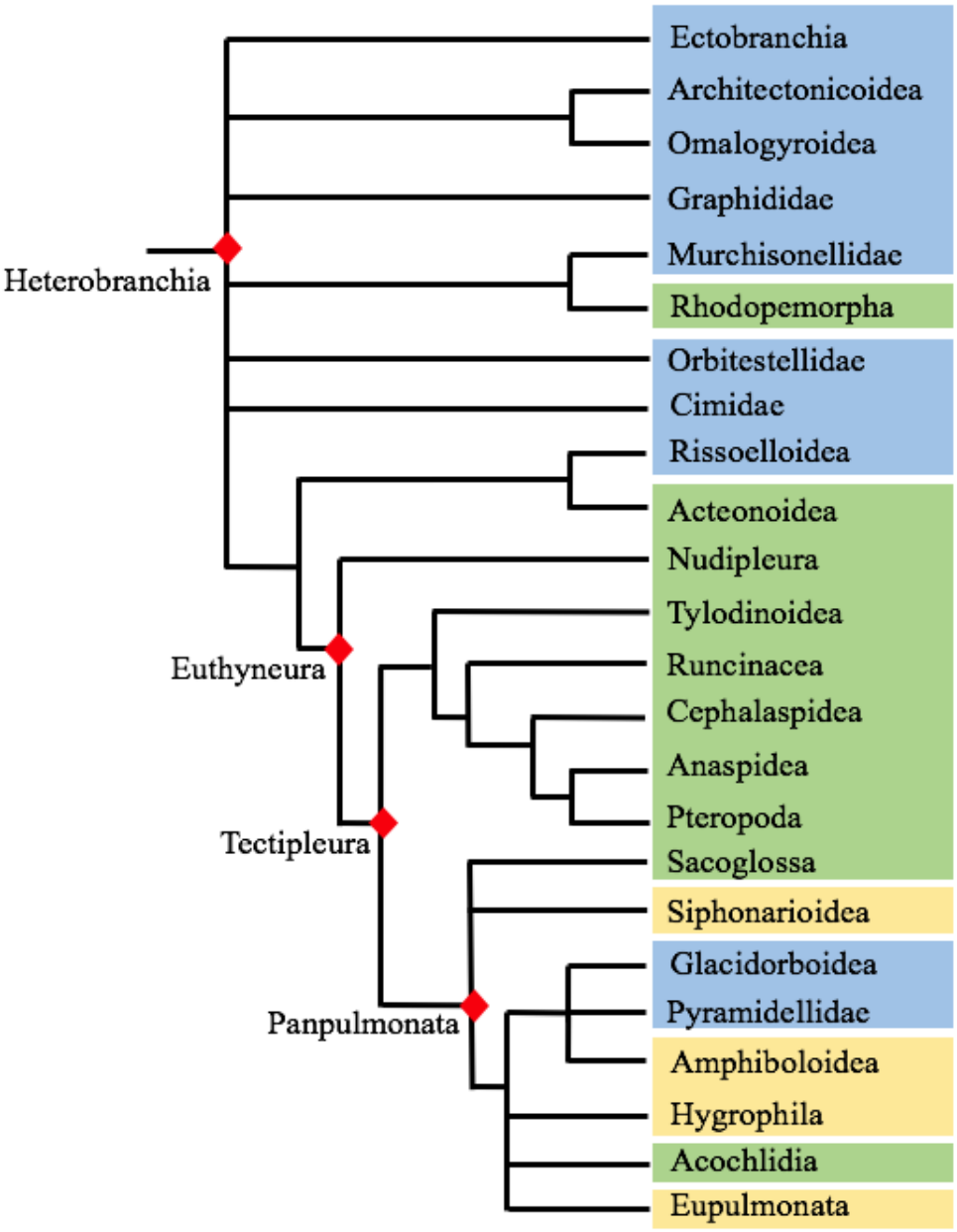

Figure 4. Recent phylogenetic hypothesis for relationships among clades within the Heterobranchia with data combined from Schrödl et al. (2011), Jörger et al. (2010), and Dinapoli and Klussmann-Kolb (2010). Red diamonds represent nodes that are well-supported. Adapted from Wägele et al. (2014). Blue represents traditional lower heterobranchs, green represents traditional opisthobranchs and yellow represents traditional pulmonates.

1.4.2 Morphology of the pyramidellid feeding system

Instead of using a radula for feeding, which is typical of most gastropods, including heterobranchs, the foregut of a typical pyramidellid begins at the anterior portion of the organism with a sharp stylet (piercing spine), which is encased in a stylet sheath. The stylet stabs through the epidermis of a host so that body fluids of the host can be pumped into the foregut of the pyramidellid. Furthermore, all pyramidellids feed by means of what Fretter and Graham (1949) identified as an acrembolic proboscis (Figure 5). This type of proboscis is essentially an introvert formed by a tubular invagination of snout body wall. When the proboscis is retracted (inverted), the external opening on the head is not the true mouth. The true mouth is carried to the proboscis tip when the acrembolic proboscis is extended (everted).

The everted proboscis of most pyramidellids has a sucker at its everted terminus, although a sucker is absent in two species of *Odostomella* (Schander et al. 1999). The sucker is used by the feeding pyramidellid to attach to its host (Peterson 1998). The sucker of the pyramidellid genera *Boonia* and *Odostomia* bears two openings: the central opening is where the stylet protrudes to impale the host, whereas the opening that is offset to the dorsal side of the stylet opening leads into an oral tube that is separate from the stylet apparatus (Fretter and Graham 1949; Maas 1965; Wise 1993, 1996). In other pyramidellids, the sucker bears only one opening, which is used as both the mouth and stylet aperture (Peterson 1998; Schander 1999) (Figure 6).

In 1949, two important papers on the anatomy of the foregut of pyramidellids were published. According to Ankel (1949a, 1949b), the stylet is formed as a derived radular tooth. Alternatively, Fretter and Graham (1949) interpreted the stylet as a modified jaw structure. Since the publication of these two papers, all subsequent mentions of the feeding system of members of the Pyramidellidae have stated that the stylet is a modified jaw, rather than a derived radular tooth. According to Maas (1965), ontogenetic information is required to determine which interpretation is correct.

Wise (1993) proposed that muscles within the stylet bulb contract in order to force the stylet out of the sheath. Stylet sheath morphology varies among species of pyramidellids; *Odostomia unidentata* has a flexible and long sheath (Fretter and Graham

1949), whereas the sheath of *O. eulimoides* is described as being short, narrow and curved (Maas 1965).

The muscular buccal pump is located posterior to the stylet apparatus and oral tube (when present) (Wise 1996; Peterson 1998) (Figure 6A). The lumen of the buccal pump is lined by cuticle and its wall includes both radial dilator and circular constrictor muscles. The dilator muscles of the pump are used to create a vacuum; fluid flows into the buccal pump through the oral tube and subsequent contraction of constrictor muscles of the buccal pump force the fluid in the buccal pump into the esophagus (Peterson 1998). Some authors have distinguished the parts of the buccal pump before and after the emergence of the esophagus as buccal pump I and buccal pump II, respectively (Figure 6B) (Maas 1965; Wise 1993; Collin and Wise 1997). However, Peterson (1998) uses the term 'buccal pump' for both parts. In *Odostomia eulimoides* and *Boonea impressa*, the cuticle-lined lumen of buccal pump I has a tri-radiate shape, whereas the lumen of buccal pump II is a flattened ellipse in cross-sectional profile (Maas 1965; Wise 1993).

In *Boonea* and *Odostomia*, the esophagus arises immediately posterior to the junction between buccal pumps I and II (Wise 1993, 1996; Collin and Wise 1997). To ensure unidirectional fluid flow, a valve-like structure is located at the opening to the esophagus (Peterson 1998) (Figure 6A). The esophagus leads to a simple stomach that receives openings of two digestive diverticula and a very short intestine leads to the anus (Fretter and Graham 1949).

Ducts from a right and left salivary gland are highly convoluted as they travel anteriorly from the glands. In members of the genera *Odostomia* and *Boonea*, ducts of the right and left salivary gland are embedded within the ventro-lateral walls of the buccal pump I as they travel anteriorly (Wise 1996). In all pyramidellids investigated to date, the left and right salivary ducts both enter the stylet bulb, whereupon they merge to form a common salivary duct (Ankel 1949b; Fretter and Graham 1949; Maas 1965; Wise 1993; Peterson 1998). The common salivary duct then extends down the hollow lumen of the stylet and opens at its tip. Two potential functions exist for the salivary glands according to Peterson (1998): lubrication for the swallowing of food and digestion assistance via the secretion of toxins or proteolytic enzymes.

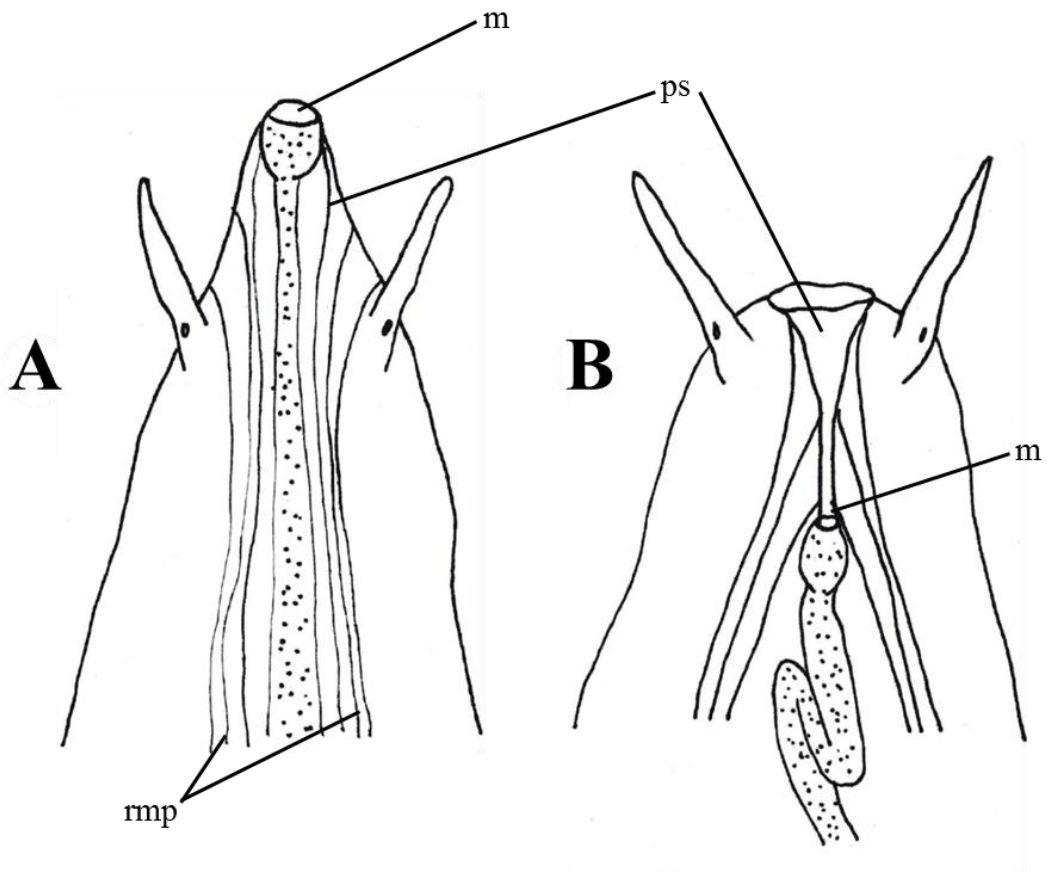

Figure 5. Acrembolic proboscis type.

A. Proboscis extended. **B.** Proboscis retracted. Gut is stippled. Abbreviation: m=mouth, ps=proboscis sheath, rmp=proboscis retractor muscles. Adapted from Fretter and Graham (1994).

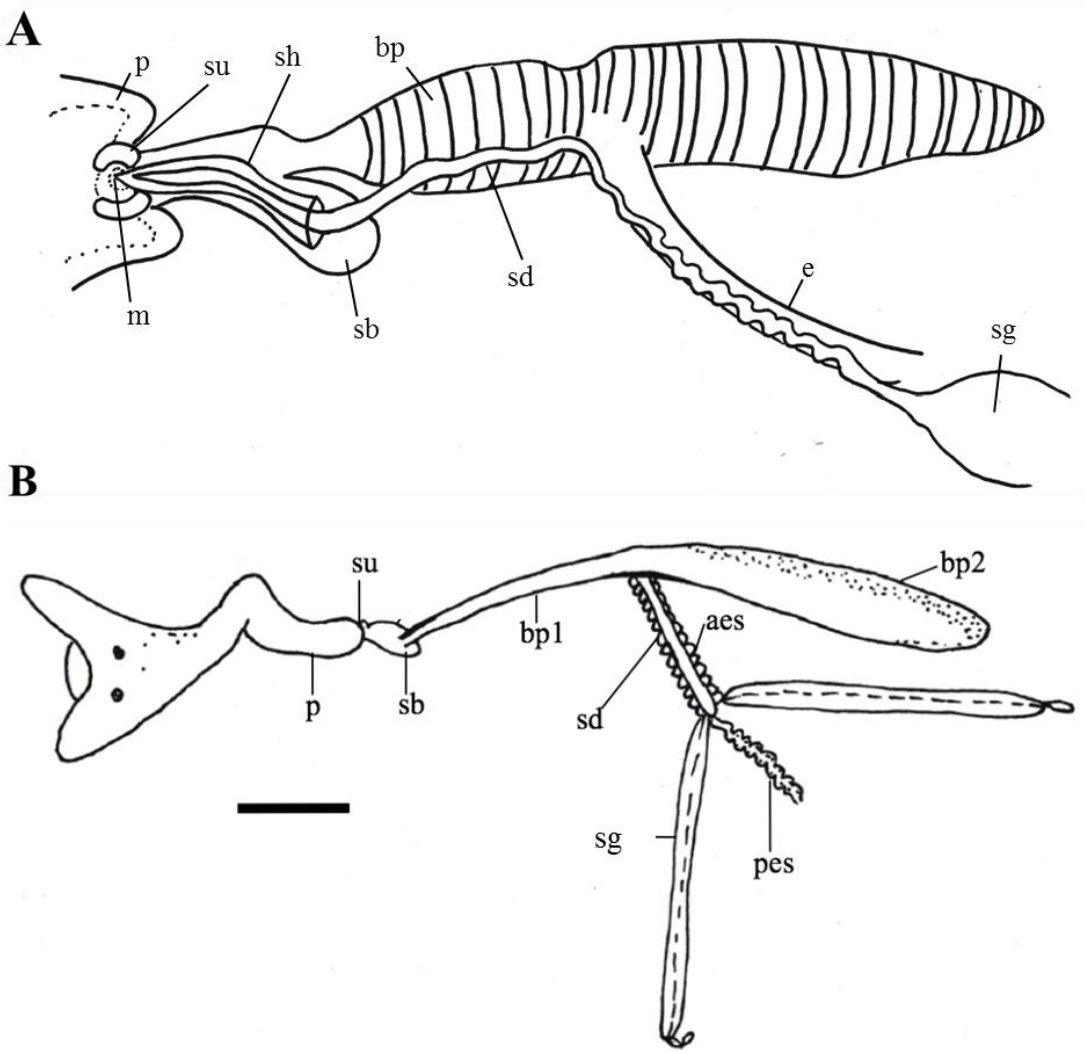

Figure 6. Sketches illustrating foregut morphology in lateral view (left to right=anterior to posterior).

A. *Sayella fusca*. Adapted from Peterson (1998). **B.** *Odostomia columbiana*. Scale bar = 500μm. Adapted from Collin and Wise (1997). Abbreviations: aes=anterior esophagus, bp=buccal pump, bp1=buccal pump I, bp2=buccal pump II, m=mouth, e=esophagus, p=proboscis, pes=posterior esophagus, sb=stylet bulb, sd=salivary duct, sg=salivary gland, sh=stylet sheath, su=sucker.

1.5 Objectives

I aimed to determine whether the piercing stylet that is used for post-metamorphic feeding by *Odostomia tenuisculpta* develops from a ventral out-pocketing (ventral module) of the larval esophagus. One hypothesis surrounding formation of the stylet is that it could be a derived radular tooth (Ankel 1949a, 1949b). There is precedent among the Gastropoda for derived radular teeth that are no longer attached to a basal chitinous ribbon and also for bizarrely shaped radular teeth. For example, the cone snail, which is a predatory neogastropod, has radular teeth yet they are found in the form of elongate, individual, hollow harpoons (Schulz et al. 2004). Another hypothesis is that the stylet is a highly derived derivative of the jaw (Fretter and Graham 1949). If the stylet and stylet sheath develop within a ventral out-pocketing of the larval esophagus, then it would be best interpreted as a homologue of a radular tooth. Alternatively, if it develops from a site along the dorsal side of the larval esophagus or post-metamorphic foregut soon after metamorphosis, then the stylet and stylet apparatus would be best interpreted as a modified jaw structure.

Odostomia tenuisculpta is a pyramidellid that hatches as a feeding larva and eventually undergoes a complex metamorphosis to become a benthic predator (Fretter and Graham 1949; Schander et al. 2003). Another question I hope to answer is how the complex post-metamorphic foregut develops in *O. tenuisculpta* without interfering with larval feeding. This question has been studied for members of the Caenogastropoda; however, members of the Pyramidellidae have yet to be studied in this context.

I used the pyramidellids as a group that is unrelated to the neogastropods, but that also show highly derived foregut structures and a large number of species, in order to test the prediction that a group like this is able to develop diverse post-metamorphic feeding capabilities because the ventral module is almost completely separate from the dorsal module of the foregut. Based on previous studies on gastropods with highly derived, post-metamorphic feeding structures, I expected to find that the dorsal and ventral modules of the foregut are isolated from one another during foregut development in the larval stage. This study is the first histological and ultrastructural study looking into foregut development and specifically foregut modularity in pyramidellids, but results will

be compared to the relatively well known sequence of foregut developmental events in caenogastropods.

2.0 Materials and Methods

Odostomia tenuisculpta were reared in the laboratory from embryo to post-metamorphic juveniles. The development of the foregut was investigated and histological sectioning of six larval and four post-velum loss developmental stages was completed. Histological sections (1 µm thickness) were examined with a light microscope. Additional analysis of developing tissues was completed with transmission electron microscopy.

2.1 Collection and culturing

Adults of *O. tenuisculpta* were collected on May 6, 2015 from the siphons of *Tresus sp*. during low tide at Patricia Bay, Saanich Inlet on southern Vancouver Island, Canada (48°26′21″ N 123°26′54″ W). Egg masses (laid by adults) were kept in the laboratory in seawater until hatching. Pre-filtered fresh natural seawater was used for culturing of eggs and larvae. To protect against heavy metal contamination of seawater, ethylenediaminotetraacetic acid, disodium salt (EDTA) was added to the seawater to make an 8.6 nM solution.

Larvae of *O. tenuisculpta* were cultured in glass custard bowls, filled with 100 mL of filtered natural seawater maintained at 12°C. The initial density of larvae was 1 larva/mL. Larvae were fed with the microalgae *Isochrysis galbana* and *Pavlova lutheri*, which were added in equal amounts to give an initial density of 2×10^4 cells/mL. This concentration was increased to 3×10^4 cells/mL after one week of larval culture. Streptomycin sulfate (50 µg/ml) was added to cultures at each culture change. Cetyl alcohol was sprinkled onto the surface of the seawater to help prevent the hydrophobic larval shells from becoming entrapped in the surface tension (Hurst 1967). Larvae were transferred to fresh culture media every two days by hand-pipetting the larvae into a custard bowl of fresh culture medium.

Larvae were induced to metamorphose at or after 40 days post-hatch (dph) by moving larvae to custard bowls of filtered natural seawater containing EDTA (as

previously mentioned) and small strips of periostracum from previously frozen *Tresus sp.* siphons. Juveniles were moved to custard bowls containing seawater and small scallops as a food source for the juvenile pyramidellids. As before, seawater was replaced in the bowls every two days.

2.2 Preparation of specimens for histological sectioning and transmission electron microscopy (TEM)

Larvae were fixed and processed for histology at: hatching and at 10, 20, 30, 40, and 50 days post-hatch (dph) (Table 1). After the loss of the larval velar lobes, animals were fixed at: 24 hours post-velum loss (hpvl) and 4 days post-velum loss (dpvl), and 10 and 20 days post-metamorphosis (dpm), according to methods established by Page (2002). Specimens were slowly anesthetized by pipetting them into small glass vials and gradually replacing the seawater with a high magnesium, low calcium artificial seawater solution (Audesirk and Audesirk 1980). At 15 - 20 minute intervals, part of the regular seawater was replaced with the high magnesium, low calcium artificial seawater solution for a total duration of 2 to 2.5 hours. Subsequently, fluid in the vial was reduced to 1 ml, the vial was placed on ice, and 3 drops of a saturated solution of Chlorobutanol in seawater was added to the vial every 90 seconds for 9 minutes. The anaesthetizing solution was then replaced with two changes of primary fixative.

The primary fixative consisted of 2.5% glutaraldehyde in Millonig's phosphate buffer (pH 7.6). Once specimens were fixed, they were stored in a 6°C refrigerator and later decalcified in a 1:1 mixture of EDTA (10% in dH_20) and glutaraldehyde fixative for approximately 2 hours. Larvae were then rinsed three times in 2.5% sodium bicarbonate (pH 7.2) and post-fixed in 2% osmium tetroxide in the bicarbonate buffer. Next, larvae were briefly rinsed in distilled water, dehydrated in an acetone dilution series, and embedded in Embed 812 (Electron Microscopy Sciences) epoxy resin.

Table 1. Summary of all ages of *Odostomia tenuisculpta* that were fixed for histological sectioning.

Days post-hatch (dph)	Assigned developmental stage	Number of individuals sectioned
Newly hatched	Stage I	5
10 dph	Stage I	1
20 dph	Stage II	3
30 dph	Stage III	5
40 dph	Stage III	4
50 dph	Stage III	3
Hours post-velum loss (hpvl)		
24 hpvl	-	3
Days post-velum loss (dpvl)		
4 dpvl	-	3
Days post-metamorphosis (dpm)		
10 dpm	-	2
20 dpm	-	3

2.2.1 Histological sectioning

Epoxy embedded specimens were mounted on metal stubs and serially sectioned using a DiATOME diamond histoknife at 1 μm thickness using a Leica Ultracut UCT microtome. Sections were placed on glass slides, dried and the tissues were then stained with methylene blue and azure II (Richardson et al. 1960). Sections were photographed using a Zeiss Axioskop compound light microscope and an attached Retiga 200T digital camera via the QCapture Pro 5.1 (QImaging) computer software. Section images were put in the correct order then edited in Adobe Photoshop CS6 adjusting for contrast, sharpness and brightness of each image.

2.2.2 Transmission electron microscopy (TEM)

In order to determine the fate of the larval esophagus and the development of the foregut through metamorphosis in *O. tenuisculpta,* ultrathin sections were cut through specimens at 24 hours post-velum loss (hpvl) and 4 days post-velum loss (dpvl). Ultrathin sections were cut using a DiATOME diamond knife at 80-90 nm thickness on a Leica Ultracut UCT microtome. Cut sections were collected on copper grids and stained with 2% aqueous solution of uranyl acetate for 1 hour, rinsed, then stained in 0.2% lead citrate for 6 minutes. A Jeol 1011 transmission electron microscope was used to examine sections. Digital images were adjusted for contrast and brightness using Adobe Photoshop CS6.

3.0 Results

3.1 *Odostomia tenuisculpta*: overview of larval and metamorphic development

Young larvae of *O. tenuisculpta* were similar to those of other euthyneuran gastropods (Page et al. 2019). Characteristics of *O. tenuisculpta* that were shared by other euthyneuran larvae included a hyperstrophic larval shell (Figure 7) and a cluster of large glandular cells adjacent to the anus, which included a gland cell containing a dark red secretory product (Figure 8A and 8B). Anal glands of euthyneuran larvae that include a pigmented component have been termed "pigmented mantle organs". Larvae also had a bi-lobed, ciliated velum for swimming and feeding on microalgae and a foot with an operculum. A pair of statocysts were located within the base of the foot. At hatching, larvae of *O. tenuisculpta* had an extremely shallow mantle cavity, which subsequently deepened along the right side (Figure 8B). Eyespots began to develop at approximately 20 days post-hatch (dph) (Figure 8A) and contractions of the larval heart began at the same time as eyespot appearance. Larvae had a single larval retractor muscle, and a complete digestive tract including a left digestive gland, small right digestive gland, stomach, and intestine. The stomach was regionally differentiated into an area lined by gastric shield that had hyaline rods embedded in the shield material, and a style sac area lined by densely packed cilia (Figure 8C). Once feeding began, the left digestive gland became coloured by algal pigments (Figure 8A) but the much smaller right digestive gland did not acquire algal pigments. Throughout larval maturation, the left digestive gland grew considerably while the right digestive gland remained small.

At approximately six weeks post-hatch, the propodium, which is a swelling of the antero-ventral area of the foot, had reached its maximal size and became densely ciliated (Figure 8D). Full development of the propodium allowed larvae to crawl; at this stage the larvae were called pediveligers. When larvae of *O. tenuisculpta* reached this pediveliger stage, they were placed in bowls of seawater with small fragments of periostracum stripped from a previously frozen horse clam (*Tresus capax*) siphon to induce

metamorphosis. The onset of metamorphosis was recognized by the visible loss of the velar lobes. This was followed by a 10-day period of explosive metamorphic morphogenesis. Other than the labial pouches, salivary ducts and glands, all post-metamorphic feeding structures of *O. tenuisculpta* (i.e. piercing stylet, sucker, and muscular buccal pumps) arose during this 10-day period. Juvenile *O. tenuisculpta* began ingesting food (the bodily fluids of small scallops) using their stylet apparatus at 10 days post-metamorphosis (dpm).

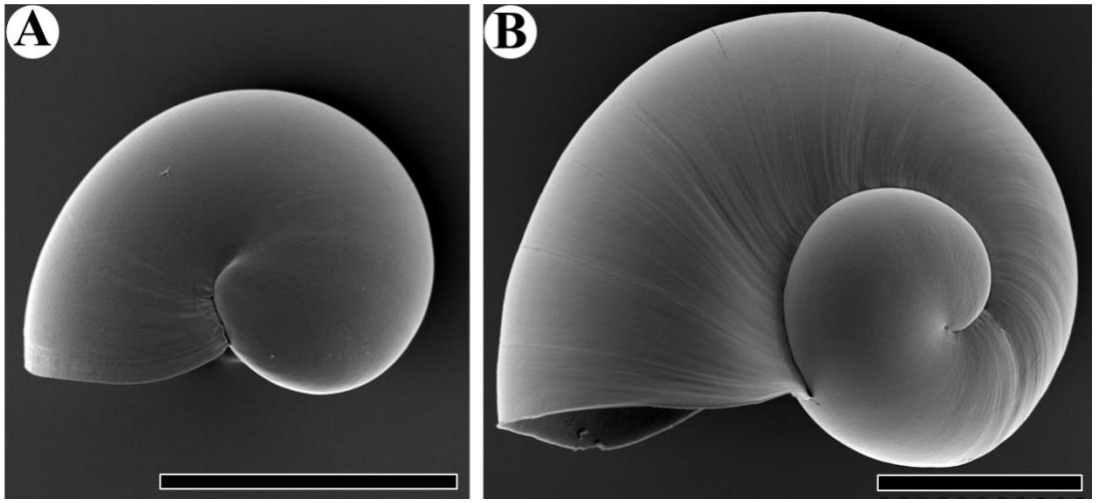

Figure 7. Scanning electron micrographs of larval shells of *Odostomia tenuisculpta* illustrating shell growth in a hyperstrophic coiling pattern. Scale bars = 100 µm. **A.** Newly hatched shell. **B.** Shell of pediveliger.

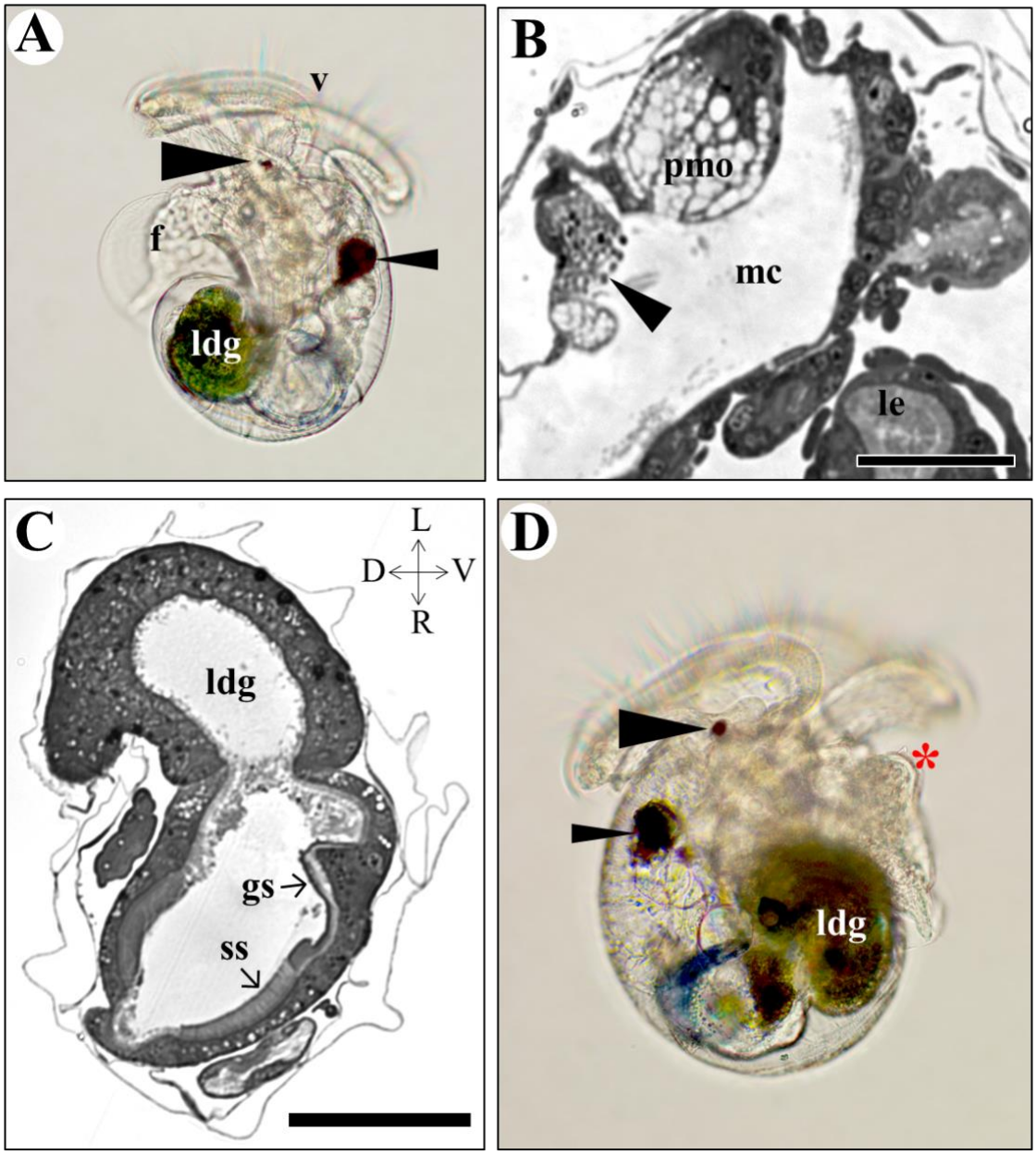

Figure 8. Veliger larval features of *Odostomia tenuisculpta*. All scale bars = 25 µm. **A.** Whole mount of a 21 dph larva; large arrowhead=eyespot; small arrowhead=pigmented mantle organ (pmo). **B.** Cross-section through a 20 dph larva showing the pmo and mantle cavity. Arrowhead indicates pmo secretions into the mantle cavity. **C.** Organs of the larval digestive system. **D.** Whole mount of a pediveliger; large arrowhead=eyespot; small arrowhead=pmo. Propodium is indicated by the red asterisk. Abbreviations: f=foot, gs=gastric shield, ldg=left digestive gland, le=larval esophagus,

mc=mantle cavity, pmo=pigmented mantle organ, ss=style sac, v=velum. Orientation axes: D=dorsal, L=left, R=right, V=ventral.

3.2 Foregut development in larvae of *Odostomia tenuisculpta*

Although development is continuous, my description of foregut development in the veliger larvae of *O. tenuisculpta* organizes the process (pre-metamorphosis) into three stages separating the important developmental events. These stages are based upon histological sections of specimens fixed over sequential larval stages.

3.2.1 Stage I

Stage I of larval foregut development began when veligers hatched out of their egg mass and continued until just before a ventral out-pocketing grew from the larval esophagus. The foregut of hatching larvae was a simple, ciliated tube (Figure 9A and 9B). At the level of the statocysts, cells along the ventral and lateral walls of the larval esophagus were larger than those forming the dorsal wall (Figure 9B). During the later part of stage I, cells within the ventro-lateral wall of the larval esophagus at the level of the statocysts increased further in both size and number (hypertrophy) (Figure 9C).

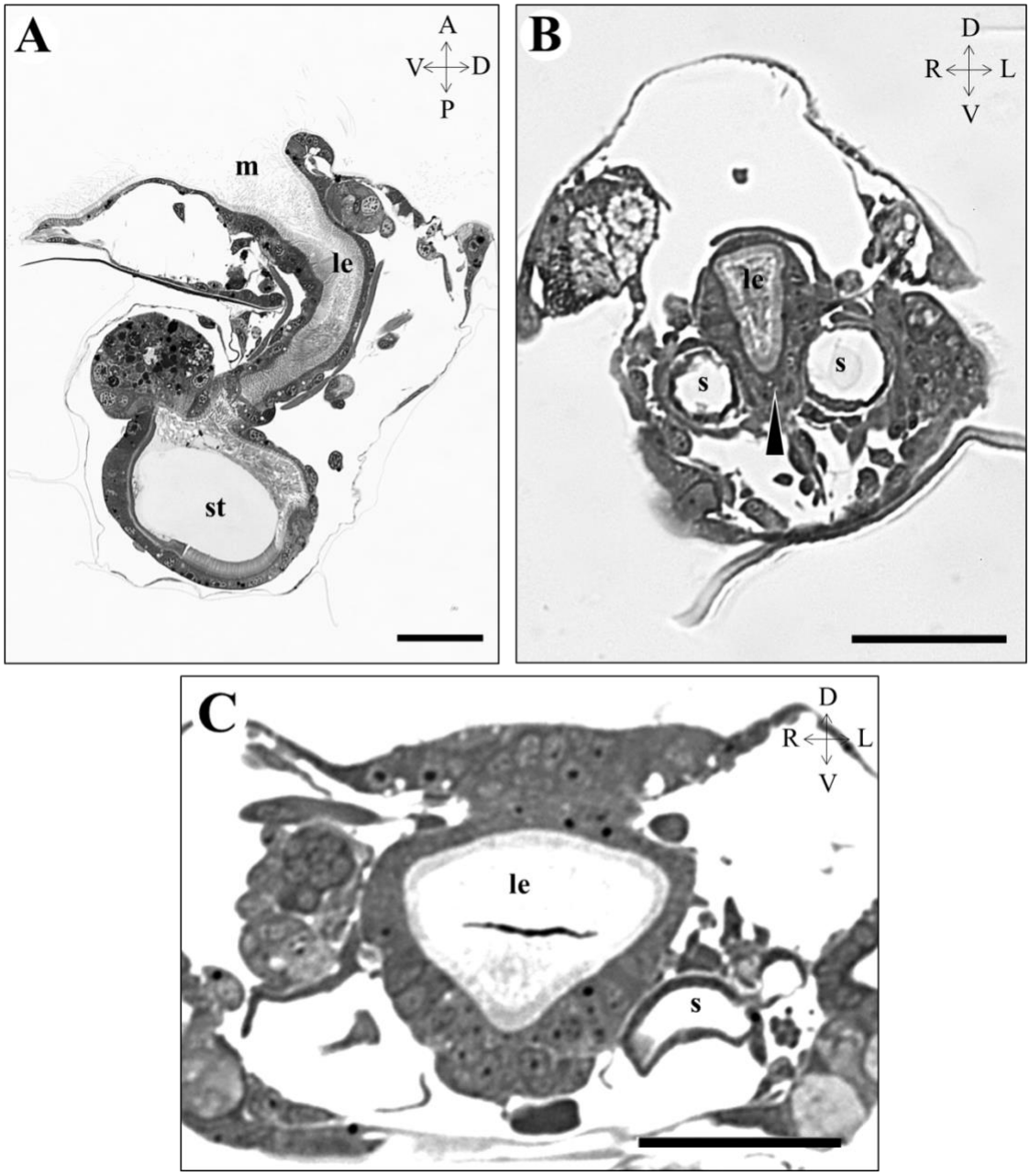

Figure 9. Histological sections through larvae of *Odostomia tenuisculpta* during stage I of larval foregut development. All scale bars = 25 µm.

A. Midsagittal, longitudinal section through a newly-hatched larva showing the larval esophagus and stomach. **B.** Histological cross-section through a newly-hatched larva showing the simple, ciliated tube of the larval esophagus. Arrowhead=large cells forming the ventral and lateral walls of the tube. **C.** Histological cross-section through the foregut of *O. tenuisculpta* at 10 dph, at the level of the statocysts. The cells of the ventro-lateral

walls of the foregut show an increase in both size and number. Abbreviations: le=larval esophagus, m=mouth, s=statocyst, st=stomach. Orientation axes: A=anterior, D=dorsal, L=left, P=posterior, R=right, V=ventral.

3.2.2 Stage II

Stage II began with the formation of a ventral out-pocketing of the larval esophagus, at the level of the statocysts, and continued until just before the formation of the labial pouches. Specimens sectioned at 20 dph, when eyespots had developed and the mantle fold had retracted, showed a ventral out-pocketing of the hypertrophied ventral cells of the larval foregut (Figure 10A). The wall of the ventral out-pocketing was made up of cells that gave rise to microvilli, but not cilia, which made them distinct from the remaining epithelial cells of the larval esophagus. As the out-pocketing grew, additional cells became clustered around the ventral and lateral walls of the esophagus in the area of the out-pocketing (Figure 10B).

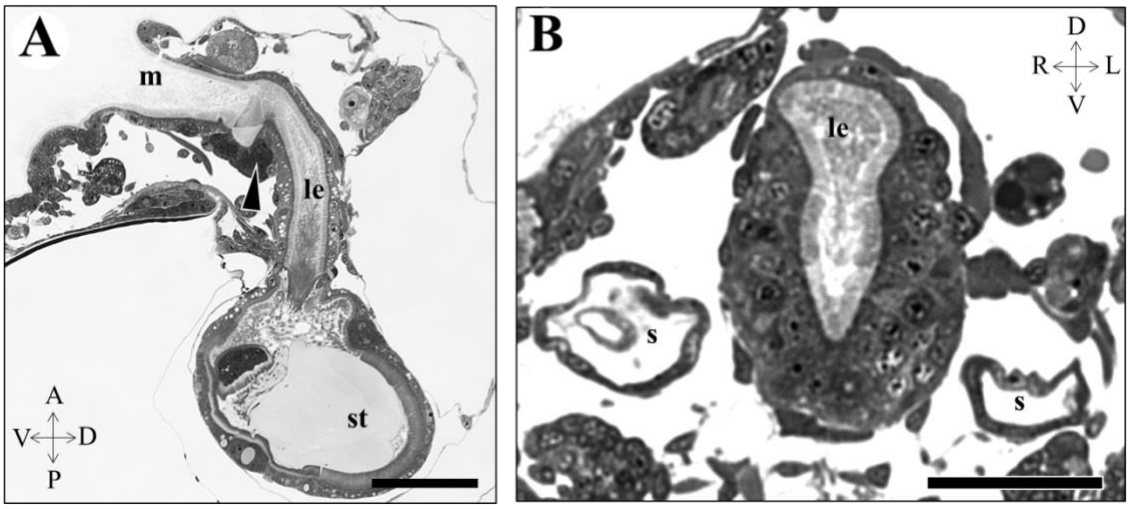

Figure 10. Histological sections through larvae of *Odostomia tenuisculpta* during stage II of larval foregut development (approximately 20 dph).

A. Midsagittal, longitudinal section of the ventral out-pocketing of the foregut, as indicated by the arrowhead. Scale bar = 50 µm. **B.** Histological cross-section through the foregut, at the level of the statocysts. Additional cells are clustered around the lateral and ventral epithelial wall of the out-pocketing. Scale bar = 25 µm. Abbreviations: le=larval esophagus, m=mouth, s=statocyst, st=stomach. Orientation axes: A=anterior, D=dorsal, L=left, P=posterior, R=right, V=ventral.

3.2.2 Stage III

Stage III began with the initial development of the labial pouches and continued until veligers became capable of crawling and metamorphic competence was reached. At 30 dph, the labial pouches appeared as a pair of out-pocketings from the ventro-lateral walls of the distal larval esophagus, just inside the larval mouth (Figure 11A). These pouches were distinctive because the apical surfaces of the cells were not ciliated; they gave rise to microvilli only. The labial pouches grew in size during the last part of larval development and eventually extended as elongate channels approximately 50 µm inward from the larval mouth opening.

Histological sections of pediveligers of *O. tenuisculpta* showed that the cells of the ventral out-pocketing from the larval esophagus had continued to increase in number, yet there was no evidence of a stylet or stylet sheath prior to metamorphosis (Figure 11B – 11D). However, cells continued to grow around the lateral walls of the larval esophagus in the area of the out-pocketing, to the point where the wall consisted of multiple layers of cells in addition to the epithelial cells that bordered the lumen (Figure 11B and 11D). The common salivary duct, which was formed by the fusion of the ducts from the left and right salivary glands, entered the posterior wall of the ventral out-pocketing (Figure 11D).

The only evidence of post-metamorphic feeding structures present in larvae of *O. tenuisculpta* were the salivary ducts and salivary glands, which were found in larvae at 30 dph and older (Figure 12A – 12C) and the labial pouches. The salivary glands were present as a pair, located on either side of the larval esophagus, but no secretory vesicles were present within the gland cells prior to metamorphosis (Figure 12C).

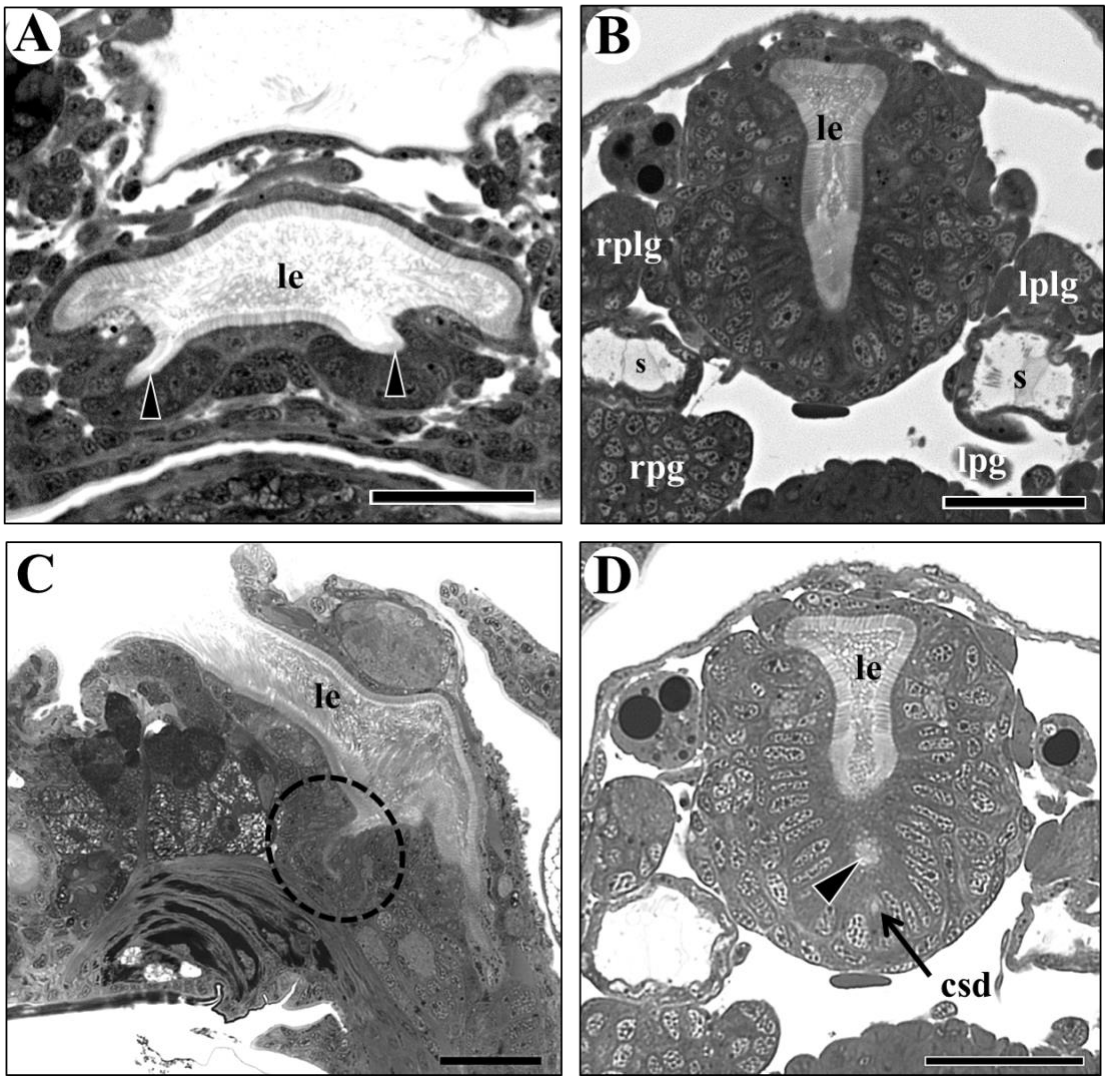

Figure 11. Histological sections through larvae of *Odostomia tenuisculpta* during stage III of larval foregut development. All scale bars = 25 μm.

A. Cross-section through the distal larval esophagus at 30 dph, with two 'labial pouches' (arrowheads) growing from the ventro-lateral area of the larval esophagus. **B.** More posterior cross-section through the larval esophagus, at the level of the statocysts. Extensive cellular hypertrophy surrounding the ventro-lateral larval esophagus is present. **C.** Longitudinal section through a pediveliger; cellular hypertrophy surrounding the ventral out-pocketing (black circle) is visible underneath the larval esophagus. **D.** Cross-section through the foregut of a pediveliger; widened lumen of the larval esophagus is visible, as well as the lumen of the ventral out-pocketing (arrowhead) and the duct of the

common salivary gland. Abbreviations: csd=common salivary duct, le=larval esophagus, rpg=right pedal ganglion, lpg=left pedal ganglion, rplg=right pleural ganglion, lplg=left pleural ganglion, s=statocyst.

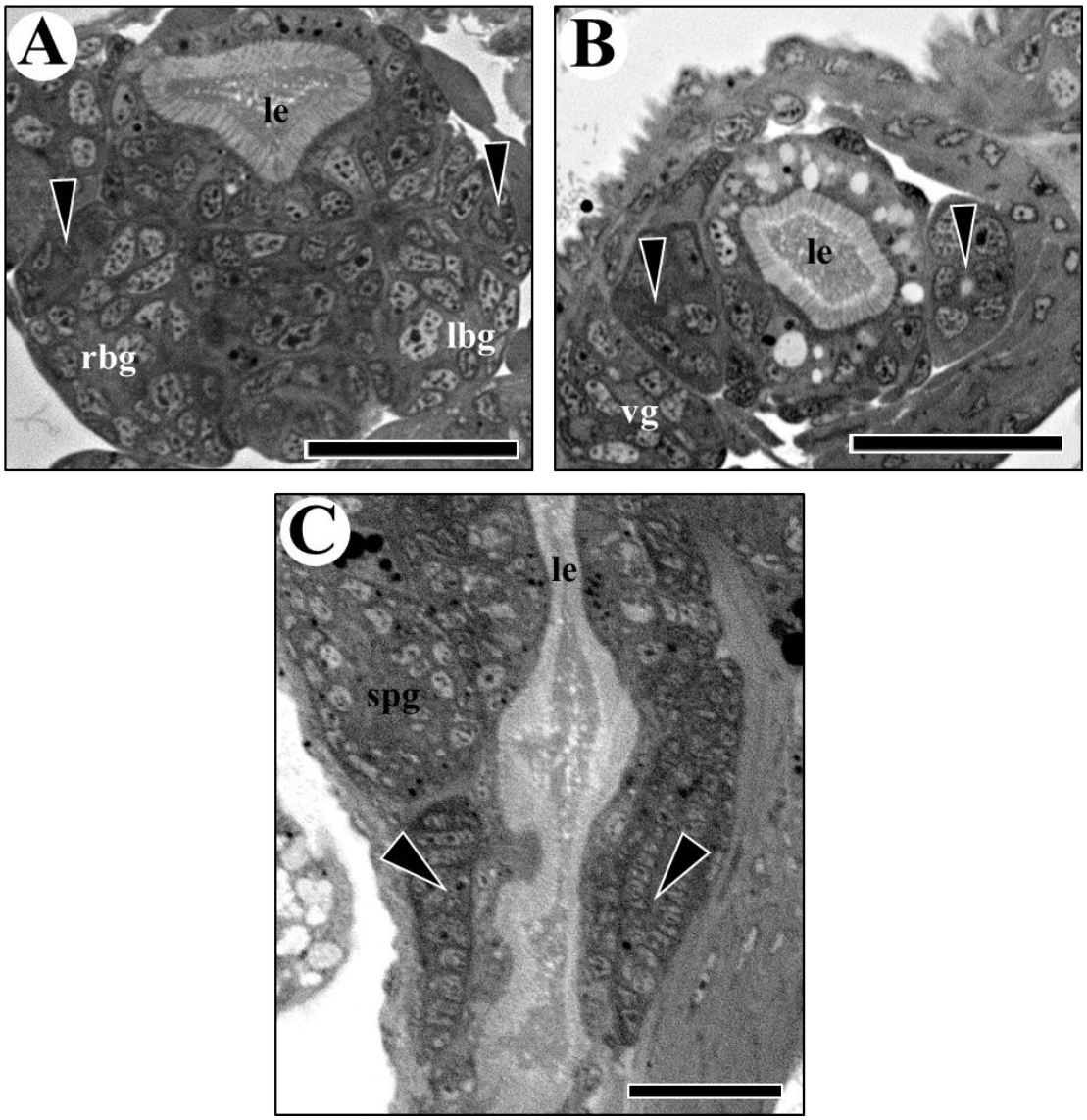

Figure 12. Salivary ducts and glands in *Odostomia tenuisculpta* at 30 and 40 dph. All scale bars = 25 μm.

A. Histological cross-section of the salivary ducts at 30 dph; the salivary ducts (arrowheads) are located on either side of the larval esophagus. **B.** Histological cross-section of the salivary ducts at 30 dph, at the level of the visceral ganglion. **C.** Histological frontal section of a larva at 40 dph showing salivary glands on either side of the larval esophagus. Abbreviations: lbg=left buccal ganglion, le=larval esophagus, rbg=right buccal ganglion, spg=supraintestinal ganglion, vg=visceral ganglion.

3.3 Foregut development during and after metamorphosis of *Odostomia tenuisculpta*

3.3.1 24 hours post-velum loss (24 hpvl)

The structure of the foregut changed dramatically during the metamorphosis of *O. tenuisculpta*. At 24 hpvl, the most dramatic visible change was the complete loss of the ciliated epithelium of the distal larval esophagus, which existed in the preceding larval stage (Figure 13A). Recall that this larval esophagus had a pair of microvilli-lined labial pouches that ran along its ventro-lateral walls, just inside the mouth opening (Figure 13A and 13B). During the first 24 hours following the loss of the velum in *O. tenuisculpta*, the ciliated, distal larval esophagus was replaced with a tube of epithelium with apical microvilli, but with no cilia arising from the apices of the cells (Figure 13C and 13D). It appeared that the cells of the larval esophagus were lost by cell dissociation and sloughed off, and that the epithelial tube that replaced the distal larval esophagus was derived from the labial pouches that developed during the late larval stage. We call this tube the "introvert tube". The external opening of the introvert tube on the head of *O. tenuisculpta* replaced the larval mouth but was in the same position as the previous larval mouth.

The introvert tube at 24 hpvl extended from its external opening on the head to the level of the nerve ring composed of the cerebral and pedal ganglia. Its lumen was slit-shaped and occasional mitotic profiles were seen (Figure 14A and 14B). In the specimen that was sectioned for transmission electron microscopy, we observed occasional ciliated cells floating free within the lumen of the introvert tube (Figure 14C and 14D). Presumably, these were residual cells, leftover from the dissociated, distal larval esophagus that were traveling down the introvert tube to more posterior parts of the digestive tract. Indeed, transmission electron microscopy showed that many phagocytized ciliated cells were present within cells of the left digestive gland.

The introvert tube eventually arrived at an area where large cells with large nuclei and prominent nucleoli were embedded in its ventral and lateral walls (Figure 14E). At this level, the lumen of the introvert tube began to take on the form of three channels: a dorsal channel and two ventro-lateral channels (Figure 14E). Study of later post-

metamorphic stages revealed that this level of the foregut would become the intersection between the introvert tube, the dorsal oral tube and the ventral stylet apparatus.

Transmission electron microscopy at a slightly more posterior level of the foregut revealed that the three channels had deepened (Figure 15A). The dorsal channel had "discoidal reticulate lamellae" within its lumen (see below), but the two ventro-lateral channels did not. The common salivary duct was embedded within the tissue between the two ventro-lateral channels (Figure 15B and 15C).

A distinctive characteristic of the velar food groove and esophagus of gastropod veligers is stacks of so-called discoidal reticulate lamellae between the cilia and microvilli of the epithelial cells (Bonar and Maugel 1982). These are shown in Figure 16A, which is an electron micrograph through the larval esophagus of the euthyneuran *Siphonaria denticulata.* The presence of these distinctive discoidal reticulate lamellae in the dorsal channel of the metamorphosing foregut of *O. tenuisculpta* at 24 hpvl (Figure 16B) indicates that the dorsal channel originated from the larval esophagus.

The dorsal channel eventually separated from the two ventro-lateral channels and the two ventro-lateral channels became the crescent-shaped lumen of the prospective stylet bulb (Figure 17A). Within the dorsal channel (former larval esophagus), more ciliated cells of the dissociating larval esophagus were visible (Figure 17A inset). A section through a slightly deeper level of the foregut revealed complete separation of the dorsal channel and posterior extremity of the prospective stylet bulb (Figure 17B). Flanking the dorsal channel (former larval esophagus) were the two salivary ducts (Figure 17C and 17D). Transmission electron microscopy of the very narrow lumen of the salivary ducts (Figure 17B) revealed circular muscle developing around the cells delineating the tiny channel of the salivary ducts (Figure 17C and 17D).

Histological and ultrastructural sections through the foregut at 24 hpvl revealed a large number of cells flanking the walls of the dorsal channel (Figure 15A, 17A and 17B), even beyond the point where the ventro-lateral channels separated from the dorsal channel. Study of subsequent metamorphic stages revealed that this area gives rise to the buccal pump I and buccal pump II; these pumps have a multi-layered wall consisting of luminal epithelium surrounded by radial and circular muscles.

Along the remaining larval esophagus, as it travelled toward the stomach, the left and right salivary ducts that flanked the esophagus connected with the salivary glands at the level of the posterior border of the buccal ganglia (Figure 18A and 18B). A few secretory vesicles were seen within the glands at this stage.

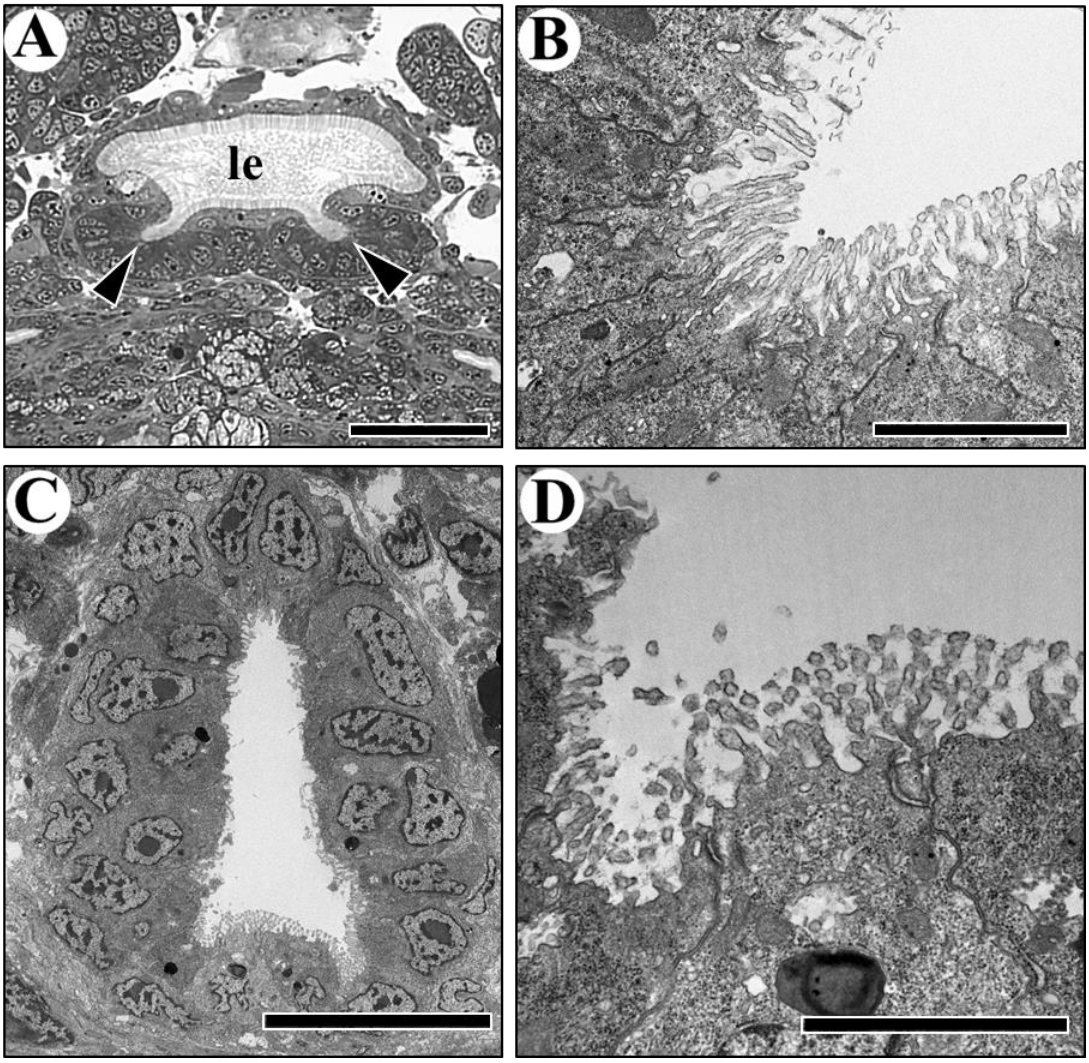

Figure 13. Labial pouches and developmental origin of the post-metamorphic introvert tube in *Odostomia tenuisculpta*.

A. Histological cross-section through the distal end of the larval esophagus at 30 dph. The two labial pouches (arrowheads) are located on the ventro-lateral sides of the larval esophagus. Scale bar = 25 μm. **B.** TEM micrograph of the apices of labial pouch cells at 30 dph; the border of microvilli is clearly visible. Scale bar = 2.5 μm. **C.** TEM micrograph of the introvert tube at 24 hpvl. Scale bar = 10 μm. **D.** TEM micrograph of the apices of introvert tube cells at 24 hpvl. The cells give rise to microvilli but not cilia. Scale bar = 2 μm. Abbreviations: le=larval esophagus.

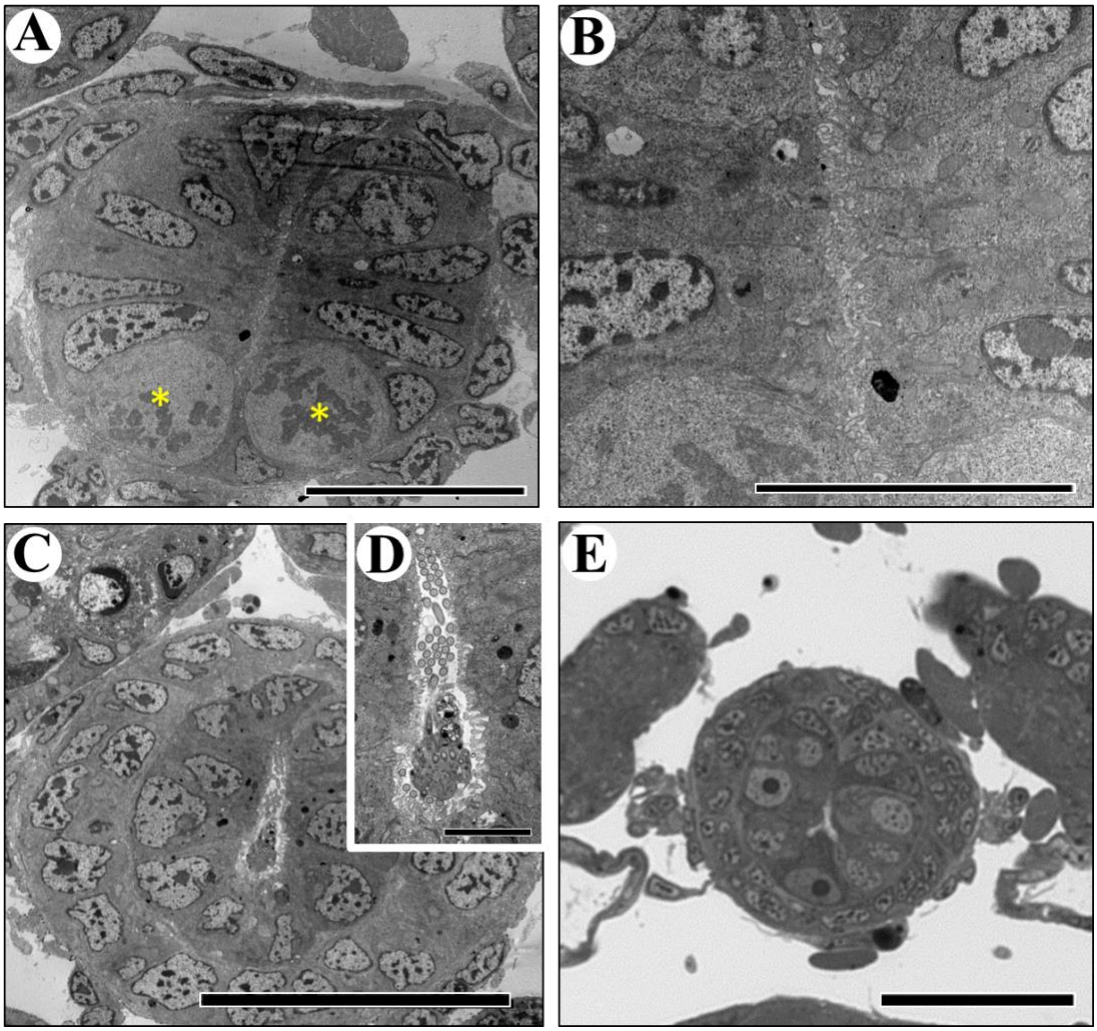

Figure 14. Introvert tube and its junction with the prospective oral tube and stylet apparatus in *Odostomia tenuisculpta* at 24 hpvl.

A. Transmission electron micrograph (TEM) of the introvert tube. Mitotic cells = yellow asterisks. Scale bar = 10 μm. **B.** High magnification TEM of the microvillus cells of the introvert tube. Scale bar = 5 μm. **C.** TEM micrograph of the introvert tube showing cells congregated around the epithelial wall. Scale bar = 20 μm. **D.** TEM micrograph showing a dissociated cell of the larval esophagus within the lumen of the introvert tube. Scale bar = 2.5 μm. **E.** Histological cross-section of the lumen of the foregut at the level where it formed a dorsal channel and two ventro-lateral channels; note very large cells embedded in the ventro-lateral walls. Scale bar = 25 μm.

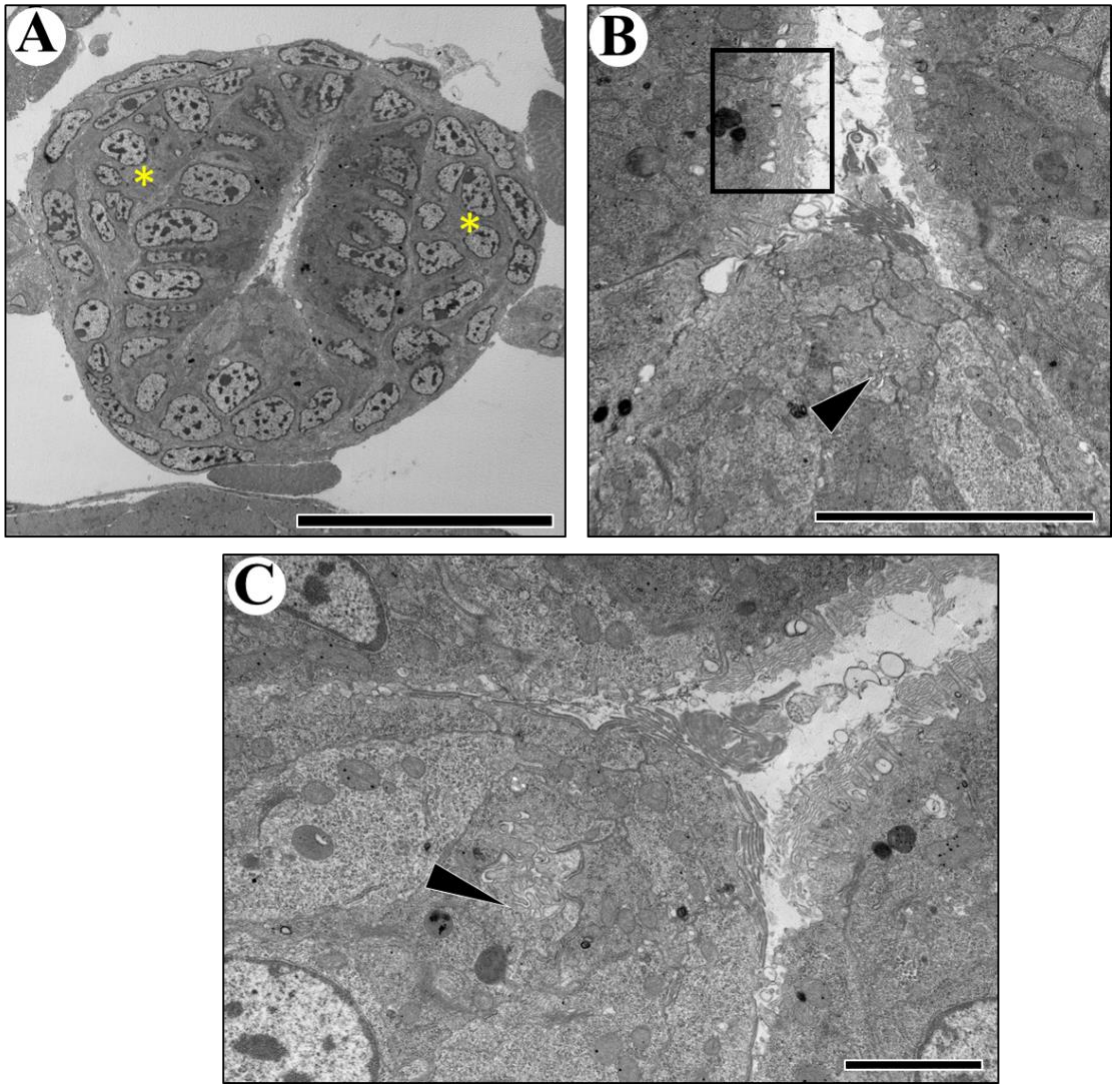

Figure 15. Dorsal and ventro-lateral channels of the foregut in *Odostomia tenuisculpta* at 24 hpvl.

A. Deepening of the dorsal and two ventro-lateral channels of the foregut lumen. Cells flanking the dorsal channel are marked by yellow asterisks. Scale bar = 10 μm. **B.** Detail from 15A showing intersection between the dorsal and ventro-lateral channels; the common salivary duct (arrowhead) is embedded in tissue between the two ventro-lateral channels. Boxed area is shown at higher magnification in Figure 16B). Scale bar = 5 μm. **C.** Common salivary duct (arrowhead) between the two ventro-lateral channels of the foregut; detail from Figure 15B. Scale bar = 2 μm.

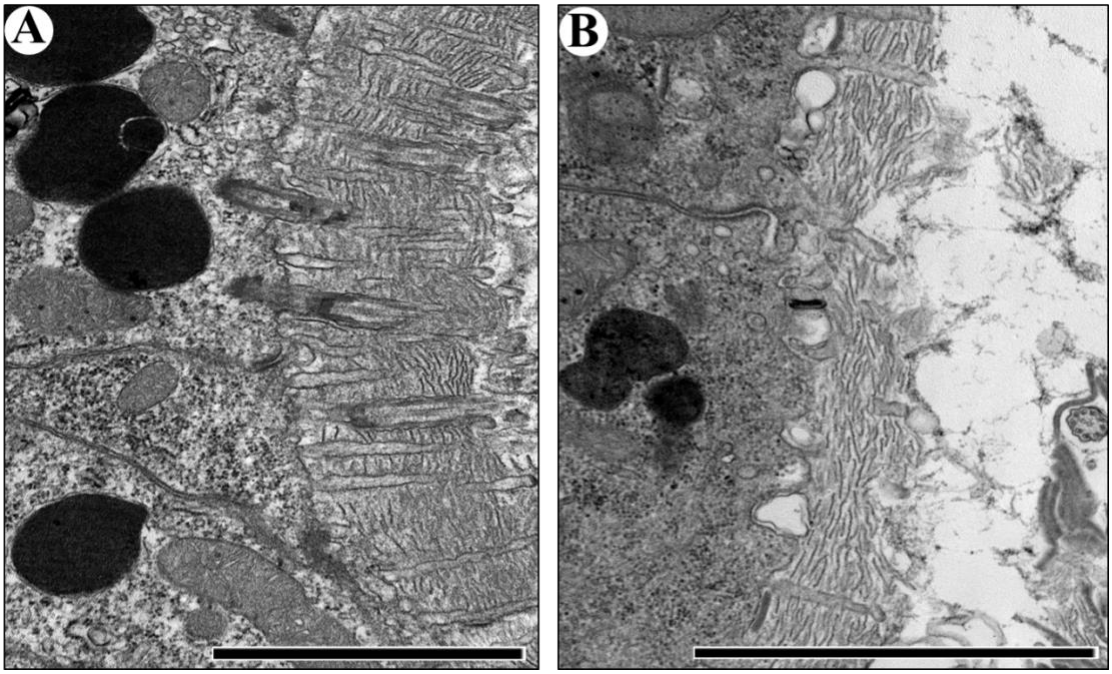

Figure 16. Discoidal reticulate lamellae. All scale bars = 2 μm.

A. Transmission electron micrograph (TEM) of the apices of the epithelial cells that line the lumen of the larval esophagus in *Siphonaria denticulata* (a typical euthyneuran pulmonate); note: cilia are present as well as microvilli and delicate strands of reticulate lamellae run between the microvilli. **B.** TEM of the apices of the epithelial cells that line the lumen of the dorsal channel in *Odostomia tenuisculpta* at 24 hpvl (detail from boxed area in Figure 15B). Note absence of cilia, but what look like discoidal reticulate lamellae extending between the microvilli.

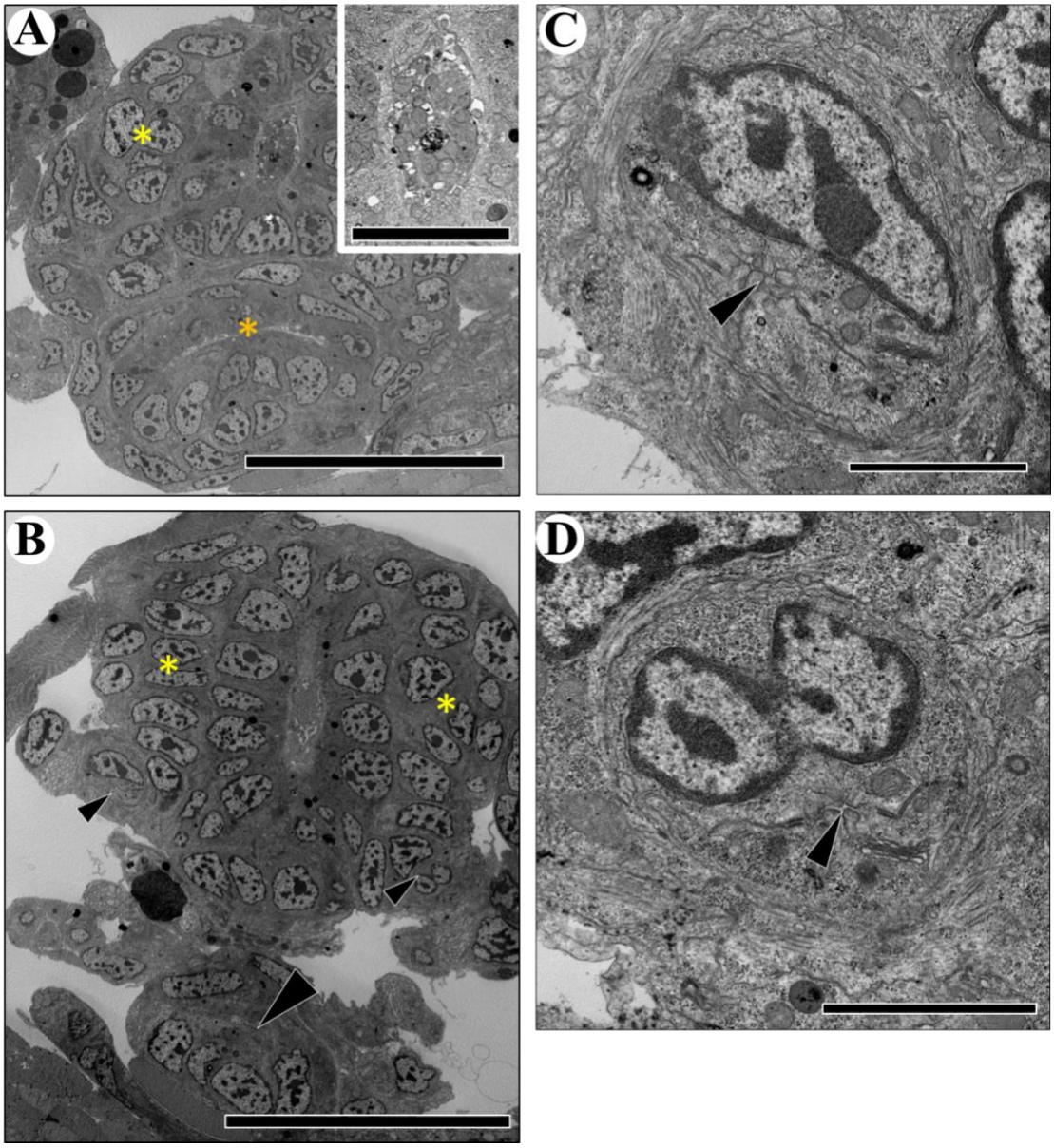

Figure 17. Prospective stylet bulb and salivary ducts in *Odostomia tenuisculpta* at 24 hpvl.

A. Transmission electron micrograph (TEM) of the stylet bulb (orange asterisk), and the dorsal channel (larval esophagus). Inset: dissociated cell of the distal larval esophagus is present within the dorsal channel. Cells flanking the dorsal channel are marked by the yellow asterisk. Scale bar = 2 μm; inset = 2 μm. **B.** TEM of a more posterior area of the foregut; the terminal extremity of the stylet bulb (large arrowhead) is visible underneath the dorsal channel. The salivary ducts are indicated by the small black arrowheads. Cells

flanking the dorsal channel are marked by yellow asterisks. Scale bar = 20 μm. **C.** TEM of the right salivary gland; lumen of the gland can be seen within (arrowhead). Detail from Figure 17B. Scale bar = 2 μm. **D.** TEM of the left salivary gland; lumen of the gland can be seen within (arrowhead). Detail from Figure 17B. Scale bar = 2 μm.

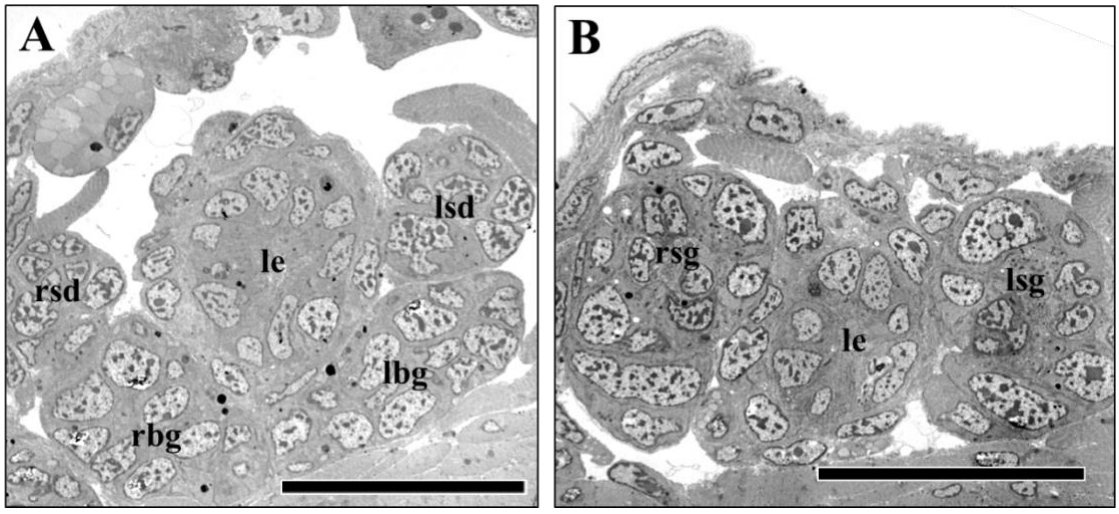

Figure 18. Posterior esophagus and salivary ducts and glands in *Odostomia tenuisculpta* at 24 hpvl.

A. Transmission electron micrograph (TEM) through the posterior end of the larval esophagus; the right and left buccal ganglia are visible. The larval esophagus is flanked by the left and right salivary ducts. Scale bar = 20 μm. **B.** TEM micrograph through a deeper level of the posterior end of the larval esophagus, now positioned in between the right and left salivary glands. Scale bar = 20 μm. Abbreviations: lbg=left buccal ganglia, le=larval esophagus, lsd=left salivary duct, lsg=left salivary gland, rbg=right buccal ganglia, rsd=right salivary duct, rsg=right salivary gland.

3.3.2 Four days post-velum loss (4 dpvl)

At 4 dpvl the introvert tube of the post-metamorphic foregut was visible between its external opening on the head to just beyond the level of the statocysts (Figure 19A) and a thin layer of cells surrounded the tube (Figure 19B). Microvilli arising from epithelial cells of the introvert were more abundant and densely packed than at 24 hpvl (Figure 19C).

The piercing stylet and stylet sheath were clearly visible at 4 dpvl (Figure 20A) past the level of the statocysts. At this level of the foregut, the former larval esophagus had become separated from the underlying stylet and stylet sheath as the presumptive oral tube (Figure 20B). The stylet and sheath were secreted by very large cells with large nuclei (Figure 21A). The lumen of the common salivary gland duct ran down the centre of cytoplasmic processes filling the centre of the stylet (Figure 21B); the lumen was tiny and recognizable by the few contorted microvilli in the centre. The base of the stylet sheath merged into the stylet sac (Figure 21C).

Once the common salivary duct exited the base of the stylet, it subdivided as the right and left salivary ducts (Figure 22), which subsequently ran along on either side of the prospective buccal pump I. The salivary ducts transitioned into large salivary glands (Figure 17 and 18). The merger of the lumen of both the right salivary duct and the common salivary duct of the stylet was visible at 4 dpvl (Figure 22A). Buccal pump I had a cuticle-lined tri-radiate lumen (Figure 22B). Buccal pump II was also visible at 4 dpvl (Figure 23).

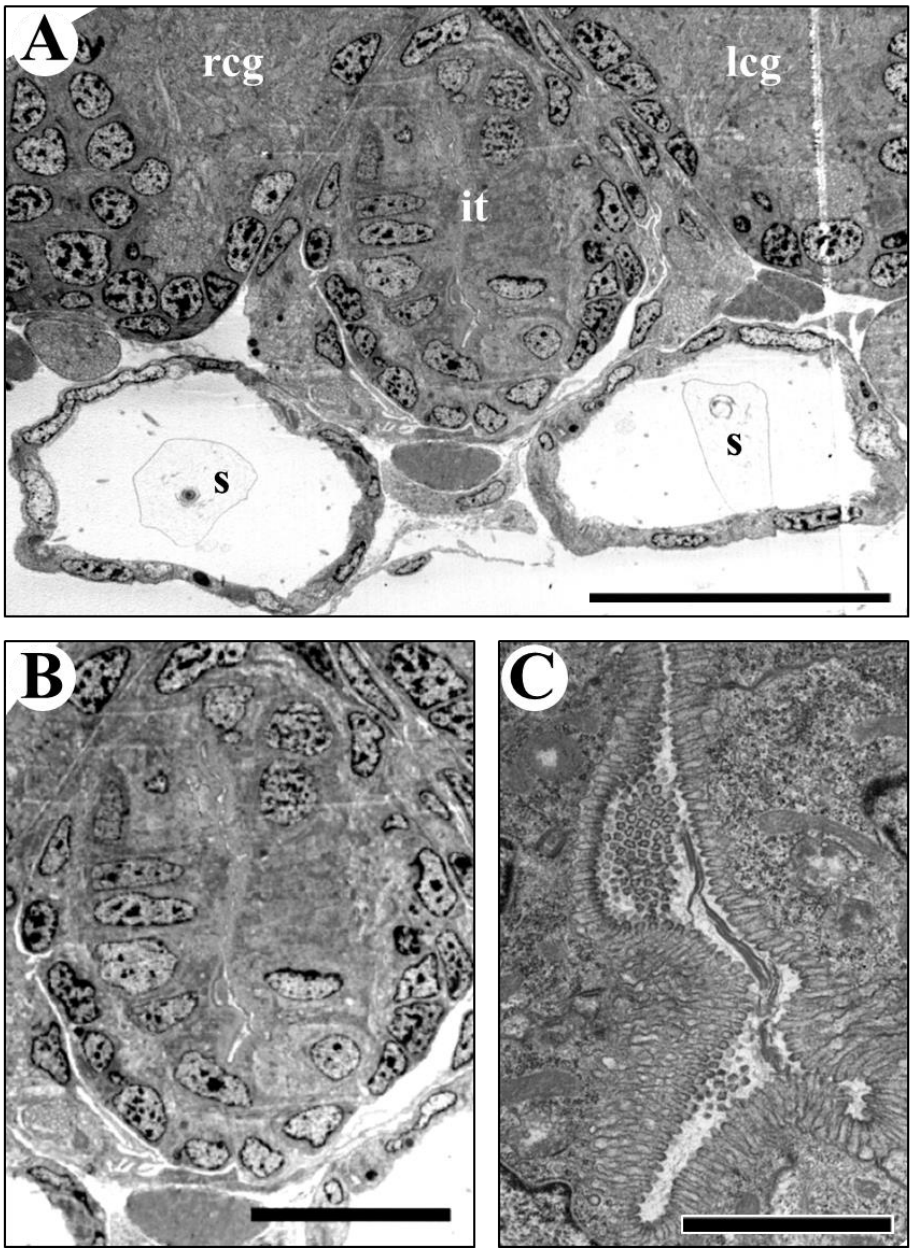

Figure 19. Introvert tube in *Odostomia tenuisculpta* at 4 dpvl.

A. Transmission electron micrograph (TEM) of the introvert tube at the level of the statocysts. The left and right cerebral ganglia are also visible in this section. Scale bar = 20 μm. **B.** TEM showing the slit-shaped lumen of the introvert tube. Scale bar = 10 μm. **C.** TEM of the introvert tube showing the densely-packed microvilli arising from the cells. Scale bar = 2 μm. Abbreviations: it=introvert tube, lcg=left cerebral ganglion, rcg=right cerebral ganglion, s=statocyst.

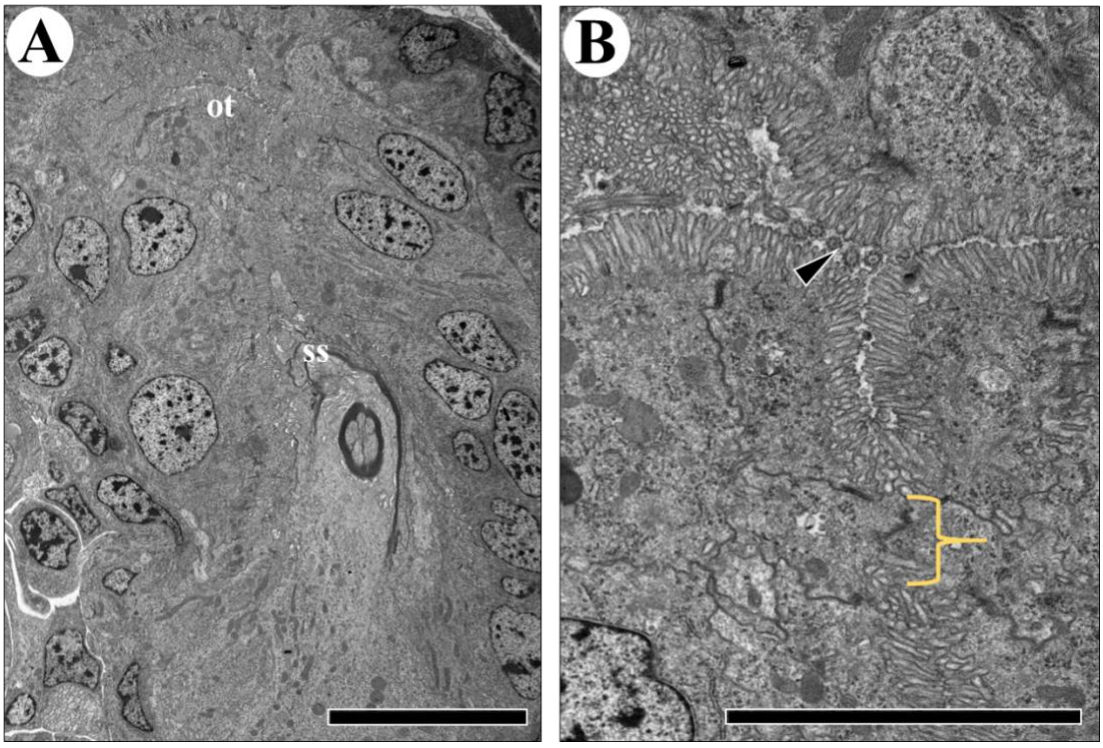

Figure 20. Morphology of the oral tube in *Odostomia tenuisculpta* at 4 dpvl. **A.** TEM showing the apex of the stylet and stylet sheath ventral to the oral tube. Scale bar = 10 μm. **B.** Detail of the oral tube from 14A (arrowhead=lumen of oral tube). Bracket demarcates the separation of the oral tube and the buccal sac. Scale bar = 5 μm. Abbreviations: ot=oral tube, ss=stylet sheath.

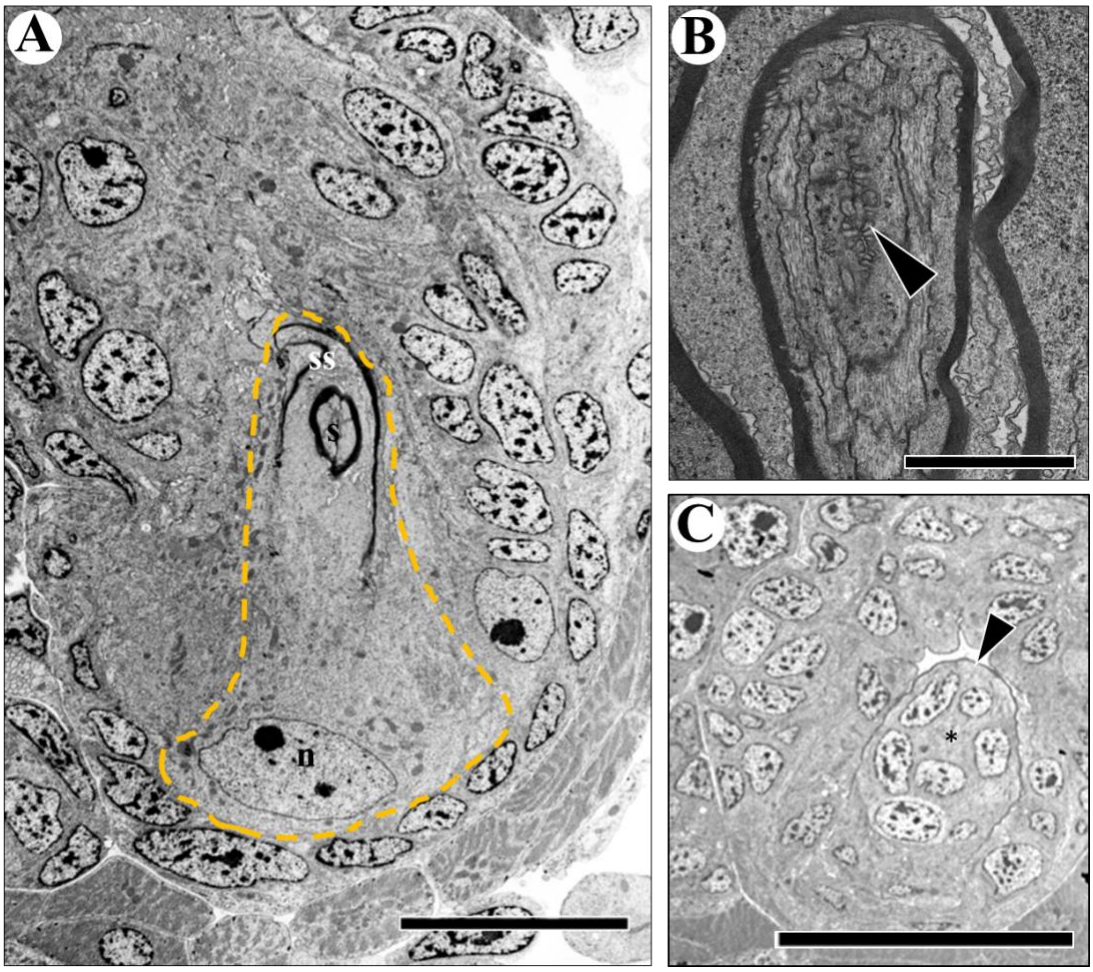

Figure 21. Stylet, stylet sheath, stylet bulb and common salivary duct in *Odostomia tenuisculpta* at 4 dpvl.

A. Transmission electron micrograph (TEM) at the level where the stylet sheath and stylet first become visible in juvenile *O. tenuisculpta*. The large cell that secretes the stylet and stylet sheath is outlined in orange. Scale bar = 10 μm. **B.** TEM micrograph of the stylet and stylet sheath. The common salivary duct of the stylet can be seen within (arrowhead). Scale bar = 2.5 μm. **C.** TEM at the anterior extremity of the stylet bulb; asterisk shows position of the common salivary duct at the base of the stylet, where the stylet sheath merges with the lumen of the stylet bulb (arrowhead=stylet). Scale bar = 20 μm. Abbreviations: n=nucleus of the stylet/stylet sheath secreting cell, s=stylet, ss=stylet sheath.

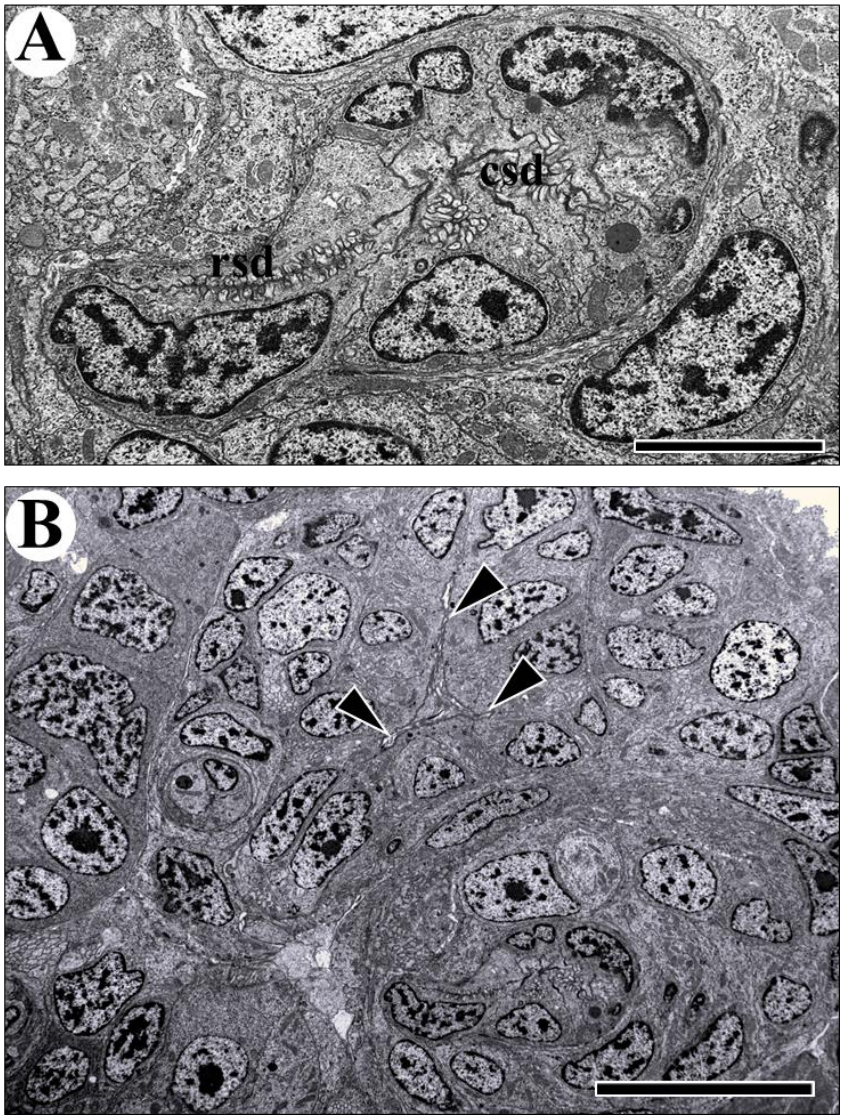

Figure 22. Right salivary gland, the convoluted right salivary duct, and the common salivary duct in *Odostomia tenuisculpta* at 4 dpvl.

A. Transmission electron micrograph (TEM) illustrating the merger between the right salivary duct and the common salivary duct. Scale bar = 2 μm. **B.** TEM of buccal pump I with its cuticle-lined tri-radiate lumen (arrowheads) and the stylet bulb. Scale bar = 10 μm. Abbreviations: csd=common salivary duct, rsd=right salivary duct.

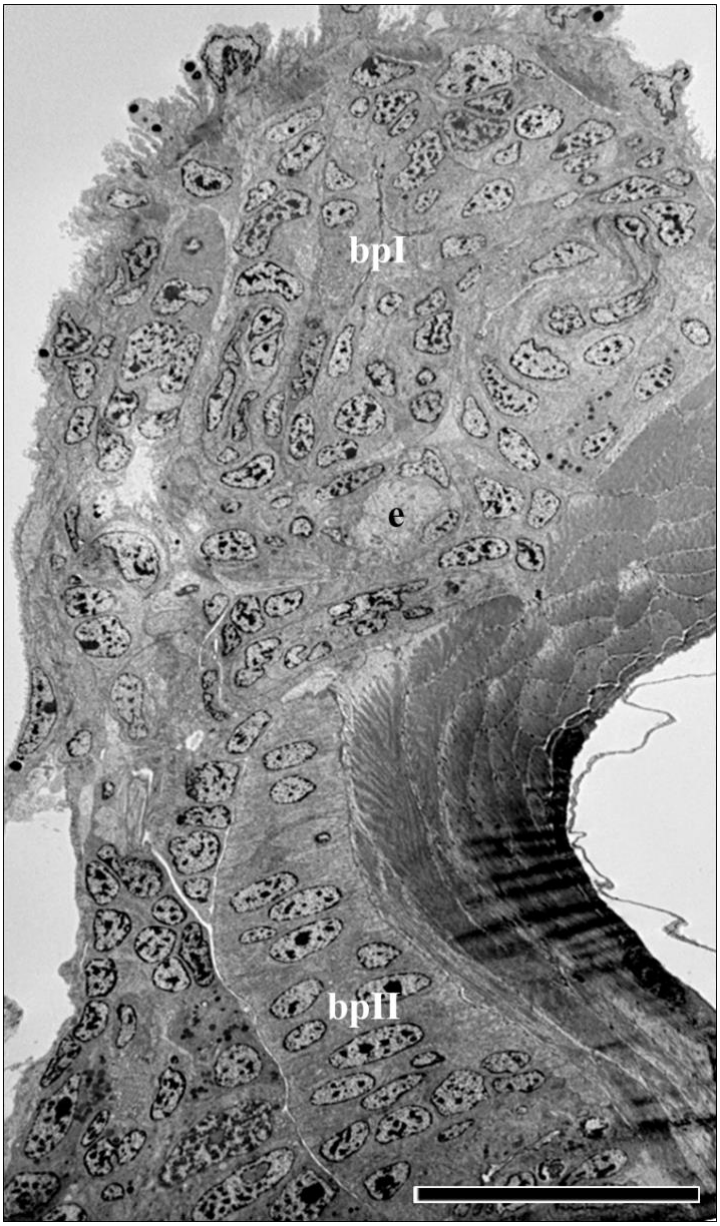

Figure 23. Transmission electron micrograph (TEM) of buccal pump I and II in *Odostomia tenuisculpta* at 4 dpvl. Scale bar = 20 µm. Abbreviations: bpI=buccal pump part 1, bpII=buccal pump part 2, e=definitive esophagus.

3.3.3 Young juveniles

At 10 dpm, juvenile *O. tenuisculpta* were able to feed and all components of the derived post-metamorphic foregut had developed and differentiated. These included the piercing stylet, buccal pump I and II, sucker and the salivary glands (Figure 24 and 25). The base of the stylet was curved as a histological section through a specimen revealed two profiles (Figure 24A). The sucker could be seen at 10 dpm (Figure 25A and 25B). At 20 dpm the same components of the derived post-metamorphic foregut were visible, yet they had increased in size (Figure 26). The larger buccal pump and salivary gland(s) were visible and cells of the salivary glands had accumulated many secretory vesicles (Figure 26A). The esophagus between the buccal pump and the stomach was long and extremely convoluted (Figure 26B).

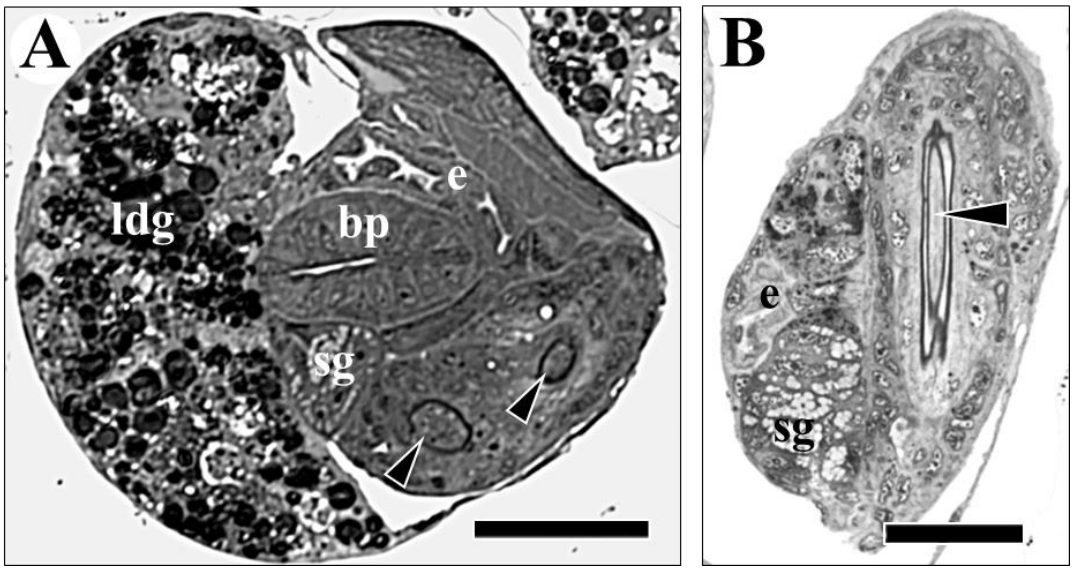

Figure 24. Components of the post-metamorphic foregut of *Odostomia tenuisculpta* at 10 dpm. All scale bars = 25 μm.

A. Cross-section showing the large left digestive gland, muscular buccal pump and two profiles through the end of the recurved piercing stylet (arrowheads). **B.** Longitudinal section through the stylet sac. The stylet (arrowhead) is visible within the stylet sheath. Abbreviations: bp=buccal pump, e=esophagus, ldg=left digestive gland, sg=salivary gland.

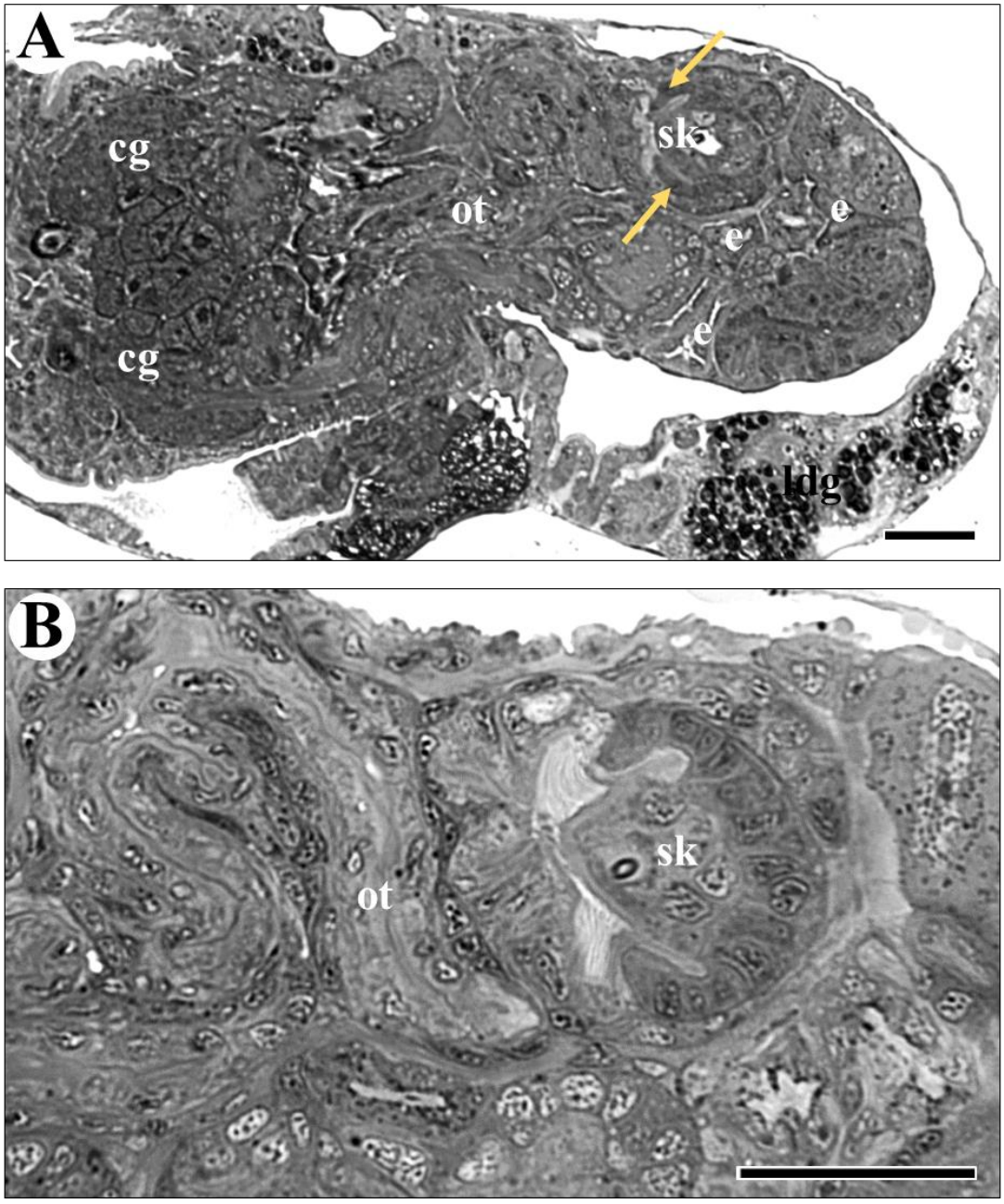

Figure 25. Post-metamorphic foregut and details of the sucker in *Odostomia tenuisculpta* at 10 dpm. All scale bars = 25 μm.

A. Cross-section through the ganglia, oral tube and convoluted esophagus. Muscle flaps of the sucker (orange arrows) were visible. **B.** Cross-section through the oral tube and sucker; detail from Figure 25A. Abbreviations: cg=cerebral ganglia, e=esophagus, ldg=left digestive gland, ot=oral tube, sk=sucker.

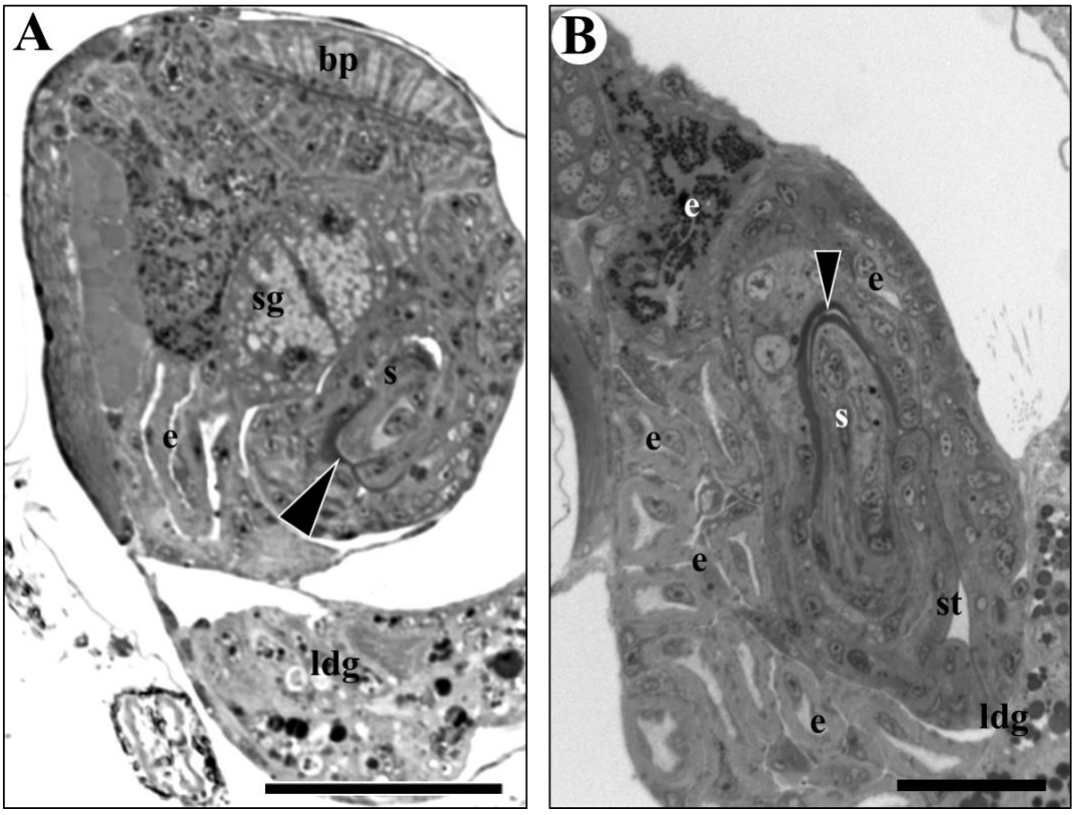

Figure 26. Components of the post-metamorphic foregut of *Odostomia tenuisculpta* at 20 dpm.

A. Cross-section displaying the larger components of the post-metamorphic foregut. Scale bar = 50 μm. **B.** Longitudinal section through the stylet and convoluted esophagus; arrowhead=stylet sheath. Scale bar = 25 μm. Abbreviations: bp=buccal pump, e=definitive esophagus, ldg=left digestive gland, s=stylet, sg=salivary gland, st=stomach.

4.0 Discussion

4.1 Homology of parts

Molluscs in the Class Gastropoda have exhibited a huge diversification in their feeding structures and food type over their evolutionary history. Members of the Pyramidellidae are a great example of gastropods that have a highly derived feeding system. The extent to which modifications have occurred is so pronounced in pyramidellids that determining the homology of various components of the foregut has been controversial. A major goal of my thesis was to examine development of a species of pyramidellid in order to gain insight into the developmental derivations of the various components of the foregut. Comparative developmental data can be valuable for inferring homologies because homologues often begin from similar developmental precursors. This comes from the fact that morphological evolution occurs via sequential changes in developmental programs. Nevertheless, development is not an infallible source of information about homologues, because development itself can evolve in ways that do not influence final morphology (Hall 1999). For example, the ancestral life-history pattern for many invertebrate groups began with a planktotrophic larval stage, but many descendant species within the various groups have secondarily lost larval planktotrophy (Strathmann 1985). This loss can result in drastic modifications of early development, because selection to maintain a functional larval body has been released. Developmental changes related to loss of a functional larval body may have no consequences to the ultimate form of the adult body. This is well illustrated by the sea urchins, *Heliocidaris tuberculata* and *Heliocidaris erythrogramma*. Adult morphology is almost identical in these two species, but profound differences in early development occur and are due to presence versus absence of a feeding larval stage (Wray and Raff 1991). Similarly, foregut development among buccinid gastropods can differ in ways that relate to presence vs. absence of a planktotrophic larval stage, not due to differences in adult morphology (Hookham and Page 2016).

Therefore, to achieve my objective of using developmental data to reconstruct the evolutionary history of foregut components in a pyramidellid it was important to study a species with a feeding larva, because pyramidellids likely evolved from an ancestor within the Euthyneura that had a feeding larval stage. I will discuss proposals about the homologues of various foregut components of *Odostomia tenuisculpta*, as inferred from comparative observations on foregut development in other gastropods.

4.1.1 Stylet apparatus

Currently, there are two hypotheses for the ancestral derivation of the piercing stylet of the Pyramidellidae, one proposed by Ankel (1949a, 1949b) where the stylet is a derived radular tooth and the other by Fretter and Graham (1949) where the stylet is a modified version of a jaw. As previously stated, there is precedence for derived radular teeth with a specialized function, such as the hollow, harpoon-like teeth of members of the families Conidae (Schulz et al. 2004) and Turridae (Ankel 1949a) that inject venom into prey.

Two observations on the development of *O. tenuisculpta* are consistent with the interpretation that the stylet complex is a highly derived radular tooth. First, the developmental site of origin for cells that secrete the stylet and sheath is similar to the developmental site of origin for the radula of other gastropods, such as the euthyneuran *Siphonaria denticulata*. Second, when the stylet and stylet sheath were first visible in animals at 4 dpvl, we also observed large cells with large nuclei that were presumably secreting the stylet and associated sheath (Figure 21A). In gastropods, radular teeth are secreted by the microvillar activity of odontoblasts in one of two ways: either by many thousands of small odontoblasts or by only a few large odontoblasts (Mikhlina et al. 2018). In pulmonates like *Lymnaea stagnalis* and *Cepaea nemoralis*, only a few large odontoblasts secrete the radular teeth (Wiesel and Peters 1978; Mackenstedt and Märkel 1987). In the case of the aforementioned pulmonates, the odontoblasts are polyploid in nature (Mikhlina et al. 2018). This agrees with observations by Haszprunar (1988), who stated that euthyneuran odontoblasts are few in number but large and polyploid in nature. The fact that the cells secreting the stylet sheath of metamorphosing larvae of *O. tenuisculpta* are very large lends further support to the hypothesis that the stylet sheath, at least, represents a derived radular tooth.

Conversely, Fretter and Graham (1949) postulated that the piercing stylet is a modified version of a jaw. In their seminal paper on the anatomy of the pyramidellid foregut, Fretter and Graham (1949) did not distinguish the stylet sheath as separate from the stylet proper. Furthermore, they suggested that the stylet aperture was dorsal to the aperture of the oral tube. This conclusion was based on an erroneous interpretation of the

position of the labial/buccal ganglia relative to the foregut. They concluded that the stylet and stylet sheath could not be a radula, as radular teeth and the radular apparatuses of gastropods form ventrally within the buccal cavity, whereas the stylet was dorsal. Maas (1965), however, determined that Fretter and Graham (1949) misinterpreted the dorsal and ventral sides of the foregut as well as the proboscis. In pyramidellid species that have two openings on their anterior end, the 'mouth' is located dorsally and the stylet aperture is located ventral to the mouth (Maas 1965; Wise 1993, 1996).

Although the 'ventral out-pocketing' in *O. tenuisculpta* does not completely separate from the dorsal module (larval esophagus) of the foregut, the ventral area could still be classified as a homologue of the developing buccal cavity. In predatory caenogastropods in which the ventral module is almost completely separated from the dorsal module during larval development, jaws develop within the buccal cavity of the ventral module prior to metamorphosis (Page and Pedersen 1998; Page 2002). In herbivorous gastropods, in which the dorsal and ventral foregut modules are broadly connected during the larval stage, jaw structures develop within the buccal cavity only after metamorphosis (Page 2000; Page et al. 2019). Furthermore, although tissue that forms the jaws is usually located dorsally within the buccal cavity of gastropods, jaw-forming tissue may also be located on the ventral side of the buccal cavity. This is the case in euthyneurans such as the nudibranch *Flabellina verrucosa* that has large opposable jaws (Mikhlina et al. 2018). Therefore, jaws develop within the buccal cavity portion of the ventral module of gastropods, and, in some species, jaw-forming cells occur ventrally within the buccal cavity. Thus, the jaw-homologue hypothesis for the stylet apparatus of pyramidellids is not in disagreement with developmental observations.

Although large cells are thought to be associated predominantly with the secretion of the radular membrane and teeth of euthyneurans, large cells also secrete the buccal hooks of the pteropod euthyneuran *Clione limacina* (Lalli 1970). According to Morton (1958) and Lalli (1970), *C. limacina* feeds by extending buccal cones that grab its pteropod prey, in order to manipulate and move it so that the shell aperture, and the body of the prey, faces the mouth. Subsequently, hook sacs evaginate to expose chitinous hooks that grab the soft tissues of the prey and bring it into the mouth of *C. limacina*, so that it can be swallowed whole. Vortsepneva and Tzetlin (2014) have more recently

argued that the hooks of *C. limacina* are jaw homologues; each is secreted by a large cell that extends microvilli into the forming cuticle of the hook.

Therefore, large cells with large nuclei that are hypothetically polyploid and secretory in nature cannot be exclusively associated with the formation of radular teeth that form from the ventral side of the buccal cavity; these types of cells have been found secreting structures from the dorsal side of the buccal cavity as well, such as the modified jaws of *C. limacina* (Lalli 1970; Vortsepneva and Tzetlin 2014).

A third potential hypothesis for the origin of the stylet and stylet sheath is that they are both derived from, or are an elaboration of the internal cuticle of the buccal cavity and associated buccal pump; the cuticular nature of the stylet (Fretter and Graham 1949) is extremely similar to that of the cuticular basal ribbon that radular teeth reside on.

4.1.2 Buccal pump I

Buccal pump I was first identifiable in specimens at 4 dpvl. It developed from a portion of larval esophagus immediately posterior to the level of the ventral out-pocketing. This area was clearly esophageal tissue retained from the larval stage because its lumen at 24 hpvl was lined with "discoidal reticulate lamellae". Discoidal reticulate lamellae are a highly distinctive and characteristic feature of the velar food groove, mouth and larval esophagus of gastropod veligers; they are not present in post-metamorphic gastropods (Bonar and Maugel 1982). Discoidal reticulate lamellae were not visible in *O. tenuisculpta* at 4 dpvl. Instead, this area of the esophagus had a tri-radiate lumen lined by cuticle.

In addition to obvious differences in the differentiation state of the foregut of late larvae of the siphonariid *Siphonaria denticulata* and the pyramidellid *Odostomia tenuisculpta*, the two also differed in the extent of cell accumulation around the lateral walls of the foregut (Figure 11). At 24 hpvl, these accumulated cells were clearly associated with the lateral walls of the retained larval esophagus and extended posteriorly well past the end of the prospective stylet bulb (Figure 15A, 17A and 17B). Sectioning and analysis of animals at 4 dpvl and 10 dpm revealed that the area where the large masses of cells were located gave rise to buccal pump I. The accumulated cells ultimately differentiate into the muscle cell layers investing this structure. Buccal pump I, in animals at 10 dpm, had a multi-layered wall of luminal epithelium surrounded by layers of radial and circular muscles, corresponding with reports of Wise (1993) and Peterson (1998) on other species of pyramidellids. The lumen of buccal pump I in *O. tenuisculpta* was tri-radiate in shape, as was found by Maas (1965) and Wise (1993).

The developmental origin of buccal pump II remains unknown, as it appeared in the specimen sectioned at 4 dpvl, but was not present in the specimen sectioned at 24 hpvl. As such, buccal pump II seems to be a *de-novo* structure, but more sectioning is required as well as the fixation of metamorphosing animals between 24 hpvl and 4 dpvl to determine exactly when, where and how buccal pump II forms within the foregut of *O. tenuisculpta*.

4.1.3 Acrembolic proboscis

According to Fretter and Graham (1949, 1994) and later authors, members of the Pyramidellidae feed by means of an acrembolic proboscis. With this type of proboscis structure, the outside opening on the head is not the true mouth; rather, it is just the opening to the proboscis lumen (introvert tube) (Fretter and Graham 1949, 1994). The true mouth, instead, is located at the base of the introvert tube and is only exposed outside of the pyramidellid when the proboscis/introvert is fully extended by eversion. Otherwise, the true mouth is retracted back into the body of the mollusc.

Our results suggest that the proboscis is an introvert tube, but it is not truly an 'acrembolic' proboscis. An acrembolic proboscis forms from the snout tissue that invaginates inward so as to carry the mouth internally. Alternatively, the proboscis-like structure of the euthyneuran genus *Hydatina* is described as an eversible oral tube (Rudman 1972) because it is presumably derived from a great elongation of the buccal tube, which is otherwise just a short entrance chamber into the buccal cavity from the mouth of gastropods. Observations on the development of the introvert tube of *O. tenuisculpta* are not consistent with its interpretation as an acrembolic proboscis; rather, its development from the distal larval esophagus suggests that it is an elongate buccal tube as proposed for *Hydatina*. The labial pouches that were first seen at 30 dph had epithelium that gave rise to microvilli, and were not ciliated like cells of the larval esophagus. The pouches were located from just inside the external 'mouth' opening to approximately 50 µm inward at the pediveliger stage. Post-metamorphic TEM images showed that the epithelium of the 'introvert tube', which replaced the distal larval esophagus, looked identical to the epithelium that made up the pre-metamorphic labial pouches: epithelial cells which gave rise to microvilli and no cilia (Figure 13). From this we believe that the 'acrembolic' proboscis is not actually an acrembolic proboscis, but rather, that it is an introvert tube formed from the labial pouches derived from the larval esophagus.

4.1.4 Sucker

One of the derived post-metamorphic feeding components in *O. tenuisculpta* was the sucker. According to the developmental stages that we studied, the sucker was a *de-novo* structure that appeared between 4 dpvl and 10 dpm, and it was located at the inner end of the oral tube (Figure 25). In pyramidellids, the sucker is used to adhere to their host/food while the stylet pierces through the skin and allows the ingestion of bodily fluids (Wise 1993). The structure appeared between 4 dpvl and 10 dpm, but we were not able to determine its developmental origin.

The sucker is formed by myoepithelial cells; not by muscle cells extrinsic to the epithelium. As such, it is unlikely to be derived from jaw muscles of other gastropods, which are formed from muscles extrinsic to the epithelium (Mikhlina et al. 2018).

4.2 Modularity

Developmental modules are integrated subsets of elements within a biological organism, which are more or less integrated with other subsets (Klingenberg 2008). Within a biological system, developmental modularity is important as it can allow developmental change to occur in different modules at different times, likely permitting change without causing ill effects on neighbouring modules (Raff 1996) or the organism itself. A great example of this is the biological phenomenon of heterochrony, whereby the development of one module can be offset spatially or temporally relative to another module(s), so as to allow evolutionary change to occur without having lethal consequences (Gould 1977). Where all gastropods have some type of temporal uncoupling between different developmental modules and the timing of the formation of different structures during their development, different gastropods undergo differing degrees of spatial uncoupling associated with their developmental modules.

Page (2000) suggested that the presence of dorsal and ventral modules within the larval foregut may have been selected because of the requirement to maintain feeding in the larval stage while the diverse post-metamorphic foregut develops in species that have indirect planktotrophic development. This is seen in herbivorous caenogastropods to a small extent and has become pronounced in predatory gastropods, in which the post-metamorphic feeding structures can develop prior to metamorphosis because of an almost complete separation between the ventral and dorsal modules of the foregut (Fretter 1969; Page 2000; Page et al. 2019).

Newly hatched larvae of *O. tenuisculpta* showed no evidence of a ventral out-pocketing (ventral module) other than slight cellular hypertrophy, which continued until the end of stage I, around the ventro-lateral walls of the larval esophagus (dorsal module). Stage II began with the formation of a small ventral out-pocketing off of the ventral larval esophagus, indicative of the ventral module. This area increased in size (by means of cellular hypertrophy) throughout stage II and culminated in stage III, when labial pouches formed in the ventral out-pocketing (ventral module) of the foregut. At this stage, the ventral out-pocketing (ventral module) was still coupled with the dorsal module

(larval esophagus) and no evidence of separation/isolation of the two foregut modules was observed prior to the metamorphic loss of the velar lobes.

I expected to see both a temporal and spatial separation of the two developmental modules of the foregut in O. tenuisculpta in pre-metamorphic individuals, as seen in other predatory gastropods with indirect planktotrophic development, but that was not the case. Rather, the only spatial dissociation/separation of the two modules was seen in post-metamorphic individuals at 24 hpvl, where the dorsal and ventro-lateral channels of the remaining larval esophagus/oral tube separated into the oral tube and the ventral stylet apparatus. In addition to the much lesser degree of spatial separation of the foregut modules than was expected, no other differentiation of the post-metamorphic feeding structures was observed prior to metamorphosis other than labial pouch formation, and the appearance of the salivary glands and ducts. This stands in marked contrast to what is known about foregut development pre- and post-metamorphosis in the Neogastropoda (caenogastropods), in which developmental modularity and a clear separation of foregut modules is present prior to metamorphosis, thus helping facilitate and speed up the transition from herbivorous to predatory feeding (Page 2000, 2005).

4.3 Juvenile drive vs. larval constraint

Many gastropods are able to undergo metamorphosis rapidly because most juvenile structures are present in the larval body prior to settlement and metamorphosis (Hadfield 2000; Hadfield et al. 2001). According to Hadfield et al. (2001), the stage when larvae have acquired incipient juvenile structures is called 'metamorphic competence'. Development of juvenile structures in the larval stage allows larvae to quickly respond to cues from the environment and undergo metamorphic morphogenesis rapidly. Feeding and swimming of planktotrophic veligers is uninhibited, whilst a fast transformation of body plan from larva to juvenile and adult modes is permitted (Hadfield et al. 2001). Hadfield et al. (2001) argued that metamorphosing larvae are vulnerable because, until juvenile structures are functional, they cannot feed or evade predators.

Herbivorous caenogastropods and heterobranchs are able to develop their post-metamorphic feeding structures prior to metamorphosis because those structures develop within a ventral buccal cavity and radular sac that does not interfere with larval feeding. Predatory gastropods, which have a much more elaborate foregut, can still develop their post-metamorphic feeding structures prior to metamorphosis because the ventral module separates almost completely from the dorsal module (larval esophagus) (Fretter 1969; Page 2000; Page et al. 2019). Pyramidellids stand out in marked contrast to the rule that gastropods with a feeding larval stage develop much of their post-metamorphic feeding structures prior to metamorphosis, despite the fact that the post-metamorphic feeding structures of pyramidellids develop from a ventral module as in other gastropods. As a result, the full metamorphic transformation of the foregut of *O. tenuisculpta* required a much longer time period, approximately 10 days, than in other temperate latitude gastropods. For example, *Trichotropis cancellata* was observed to feed between several hours to several days after metamorphic velum loss (Parries and Page 2003) and *Neverita* (=*Polinices, =Euspira*) *lewisii* and *Nassarius mendicus* were both capable of feeding at 3-5 days after metamorphic velum loss (Pedersen and Page 2000; Page 2005). All of these gastropods, including *O. tenuisculpta*, live in the coastal waters around southern Vancouver Island.

During larval development of *O. tenuisculpta*, the foregut underwent only a few small changes in preparation for metamorphosis and juvenile life, contrary to what is seen in other euthyneuran pulmonates, like *Siphonaria denticulata* (Page et al. 2019). Interestingly, stage III began with the formation of non-ciliated labial pouches, one on either side of the ventro-lateral larval esophagus, and continued until the pediveliger stage was reached. At this stage, which was close to the onset of metamorphosis, we expected to see more juvenile-specific structures, yet none (other than the salivary ducts and glands) were observed, whereas larvae of *S. denticulata* had a large ventral out-pocketing and a ribbon of radular teeth present within (Page et al. 2019). *Odostomia tenuisculpta* had none of these elaborate structures; there was no additional indication that the stylet, stylet sheath, and other derived post-metamorphic structures would be forming during the metamorphic transition. Presumably, the very elongate, dagger-shaped stylet and stylet sac can simply not be accommodated within a functional larval body. Similarly, structures of the muscular buccal pump, designed for suction feeding, cannot co-occur with structures needed for larval feeding that depend on ciliary transport.

4.4 Summary and future research

This study was the first histological and ultrastructural examination into the development and modularity of the foregut in a pyramidellid gastropod. Contrary to what we expected, *O. tenuisculpta* does not have an obvious spatial uncoupling of the dorsal and ventral foregut modules during larval development, which was previously seen during the larval development of predatory caenogastropods that feed with a proboscis (Page and Pedersen 1998; Page 2002, 2005). Dorsal and ventral foregut modules are recognizable during the development of *O. tenuisculpta*, but there is little physical separation between the two during the larval stage. Surprisingly, other than the labial pouches and the salivary glands and ducts, the derived post-metamorphic juvenile and adult feeding structures, including the stylet and stylet sheath and buccal pump I and II did not appear prior to metamorphosis, and instead arose during a 10-day period of explosive metamorphic morphogenesis after the loss of the velar lobes.

We are still unsure whether the stylet is a derived radular tooth or modified jaw structure, as the two hypotheses for the derivation of these pyramidellid structures have proposed. Alternatively, the stylet and stylet sheath could be a simple elaboration of the cuticle. Our data clearly showed that buccal pump I is derived from the larval esophagus immediately posterior to the ventral out-pocketing that becomes the buccal sac and stylet bulb of the juvenile/adult. Comparative studies and studies of additional stages of metamorphosis by *O. tenuisculpta* are required to more critically evaluate hypotheses regarding the homologies of the stylet and the acrembolic proboscis, and the origin of the sucker and buccal pump II. Studies on gene expression during pyramidellid development might also help resolve the debate about the homology of the stylet and stylet sheath. Recently, Hilgers et al. (2018) reported expression of a suite of genes within the tissue of the radular sac, which was different from genes expressed in the foot and mantle. However, gene expression by jaw-secreting tissue was not studied. It would also be revealing to undertake a comparative study on development of the foregut of *Hydatina physis* (Rudman 1972), because this euthyneuran species has an eversible oral tube, which has not been interpreted as an acrembolic proboscis.

While many of the features of *O. tenuisculpta* are not unique among different groups of gastropods, as mentioned above, members of the family Pyramidellidae have many features that require further study. Only from further investigation of additional pre-metamorphic and juvenile stages, especially between 24 hpvl and 10 dpm, can the developmental origin of the sucker and buccal pump II be completely understood.

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